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Original Articles

THE ROLE OF THE CARRIER IN THE FORMATION OF COMPLETE ANTIGENS

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NUMEROUS chemical substances produce skin sensitizations of the delayed type with great regularity upon epidermal or intradermal application to guinea pigs; among the particularly strong sensitizers are primulin, paraphenylenediamine, phenylhydrazine, pieryl chloride, 2,4-dinitrochlorobenzene, and others. However, sensitization by the epidermal route leads not only to the delayed type of hypersensitiveness, but also, as Landsteiner and Chase[1] and Chase[2] have demonstrated, to allergies of the immediate or anaphylactic type, which is demonstrable by challenge with the corresponding hapten-protein complex.

Parenteral administration of these same substances, as for instance by intraperitoneal or intravenous injection, under careful avoidance of contact with the epidermis, produces regularly sensitizations of the immediate type, but only occasionally sensitizations of the delayed type. However, if the parenteral preparation is accompanied by a simultaneous administration of certain unspecific adjuvants, particularly killed mycobacteria suspended in paraffin oil (Freund's adjuvant) or certain mycobacterial waxes, the intensity and the frequency of epidermal sensitizations increase considerably. Using mycobacteria, for instance, as the adjuvant, sensitizations of the delayed type in increased number and intensity were obtained when guinea pigs were sensitized to pieryl chloride,[3] egg albumin,[4] and tuberculin.[5]

Landsteiner and Chase[1, 2] have proposed various explanations for this delayed-reactivity-promoting action of tubercle bacilli or their lipopolysaccharides, such as an improvement of the antigenic effect, an enhancement of sensitization, or a modification of the response to antigens. Raffel[4, 5] believes that the adjuvant brings about an actual modification of the allergic response.

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We have never, in all our previous studies of experimental sensitizations, found an adjuvant capable of effecting any qualitative change of an allergic response, particularly a substitution of the delayed type of sensitization for the immediate type. One can readily observe skin sensitizations of the delayed type after the parenteral injection of sensitizing agents in the absence of adjuvant, although in smaller numbers and lesser strength than in the presence of appropriate adjuvants. Tuberculin alone is likewise capable, under favorable conditions, of producing typical tuberculin hypersensitivity of the delayed type.

In a previous publication, the following explanation of the sensitization-promoting effect of certain adjuvants was proposed[6]:

All chemicals of low molecular weight which are frequent causes of skin allergies, such as picryl chloride, paraphenylenediamine, and many other simple chemicals, are haptens which become complete antigens and true sensitizers only when they are attached to carriers of high molecular weight. Most of these haptens, or their metabolites, are chemically reactive and firmly combine with proteins—in fact, with any kind of protein they may encounter at the point of introduction into the body. When a hapten is injected into the skin, it there encounters two predominant types of protein, namely, collagen and keratin (and their precursors), both of which are fibroid and rigid proteins with which the hapten will combine, often under formation of cross-links. If, however, this same hapten is injected intraperitoneally, it will come in contact with very little collagen and no keratin at all. It will, however, encounter almost exclusively the globular proteins, albumins and globulins, and will combine with them to form complete antigens.

The presence of tubercle bacilli and waxes in the peritoneal cavity and in close proximity to the haptens provides the hapten with collagen and thereby enables it to form complete antigens containing fibroid proteins. Mycobacteria and their waxes produce at the site of deposition tubercles composed of epithelioid and giant cells. In these tubercles, collagen is formed and accumulated at a very early stage. In the presence of mycobacteria, collagen is thus accumulated at sites which are normally very low in collagen content (the normal collagen content of the organs is about $\frac{1}{20}$ that of skin). If, therefore, picryl chloride is injected intraperitoneally together with a tubercle-producing adjuvant, it will encounter in its quest for a carrier with which it can combine to form a complete antigen not only the proteins which are normally abundant in the peritoneal cavity, namely, albumins and globulins, but also collagen which it normally would find in abundance only in the skin.

It is therefore likely that the chemical composition of complete antigens formed after penetration of a hapten into the skin, the peritoneum, or any

organ in the presence or absence of mycobacteria is not the same since the proteinic carriers, to which they become attached, are different. They unquestionably have different solubilities, rigidities, and shapes. Consequently, one may expect that the antibodies formed by the presence of these diverse complete antigens are likewise different.

According to accepted theories, the specific antibodies are fabricated on the templates of the protein-synthesizing enzymes modified by the presence of specific antigens, and are complementary in shape and form to the specific antigens. Thus, it may be expected that an antibody formed on a template modified by an antigen containing globular proteins as the carrier will be different from an antibody molded on templates modified by the presence of complete antigens containing fibroid proteins as the carrier. One may anticipate that the introduction of haptens into the peritoneal cavity normally induces the development of humoral antibodies of the anaphylactic or "immediate type" of allergy, while in the presence of collagen in the tubercle sessile antibodies of the "delayed reaction type" are fabricated in addition to the humoral antibodies. This theory would explain why the injection of a hapten directly into the peritoneal cavity is usually followed by the development of a hypersensitivity of the immediate type, while the injection of the same hapten into the peritoneal cavity together with a mycobacteria-containing adjuvant will favor a considerable increase in number and severity of the reactivities of the delayed type.

We have tested this hypothesis by substituting, as an adjuvant, mycobacteria or their waxes by a purified collagen preparation (procollagen). As previously reported,[6] guinea pigs injected parenterally with picryl chloride in the presence of procollagen as the adjuvant reacted after an appropriate sensitization period upon epidermal challenge with delayed reactivities in increased number and strength; the delayed-reaction-promoting effect of the procollagen adjuvant was of the same magnitude as that obtained with Freund's adjuvant containing mycobacteria. In the present article, we report on additional experiments in which guinea pigs were sensitized to picryl chloride or Old Tuberculin by parenteral application with and without the addition of different "adjuvants," such as mycobacteria, procollagen, crystalline albumin, and paraffin oil.

EXPERIMENTAL

1. *Animals.*—In all experiments guinea pigs weighing approximately 250 grams were used.

2. *Antigens.*—Solutions of recrystallized picryl chloride were prepared in saline with the aid of absolute alcohol according to the method of Landsteiner and Chase.[1, 3] The doses used for sensitization were 0.2 mg. picryl chloride dissolved in 0.5 ml. of diluent. Procollagen, prepared as described by Orekhovich and associates,[7] was dissolved with 1:10,000 formic acid to form

5 per cent semisolutions and then emulsified with the oily components of Freund's adjuvant. The composition of the procollagen adjuvant used was as follows:

Procollagen solution, 5.0%	40 ml.
Tween 80	0.5 ml.
Dihydrostreptomycin	20 mg.
Penicillin	21 mg.
Bayol F	100 ml.
Arlacel C	4 ml.

The final approximate concentration of procollagen in this adjuvant* preparation was 1.37 per cent. (It is probable that the procollagen may have been partially transformed into gelatin during the preparation of the adjuvant.) An "albumin adjuvant" containing approximately 1.4 per cent crystallized albumin was prepared in an identical way as the above-mentioned "procollagen adjuvant."

Complete Freund's adjuvant was obtained from the Difco Laboratories.

3. *Method of Injection.*—In order to avoid contact with the skin, injections were made as first reported by Landsteiner and Chase[1] by first slitting the abdominal skin of the guinea pigs and introducing the needle slantwise through the musculature, with the use of all precautions noted by these authors.

4. *Challenge.*—Four weeks after the first sensitizing injection, the animals were challenged by (a) a single, percutaneous application of one drop of 1.5 per cent picryl chloride dissolved in olive oil, spread over approximately a 10 sq. cm. area on the right shaved flank, or (b) intradermal injection of 0.1 ml. of a saline solution containing 20 mcg. of picryl chloride. In the case of sensitization to Old Tuberculin, the challenging dose was administered by the intradermal injection of 0.1 ml. of 1 per cent and 10 per cent Old Tuberculin, diluted in saline.

RESULTS

We have performed eight experiments thus far, in which the conditions of the sensitizing procedures were varied; of these experiments, three are described hereafter.

1. In the *first experiment,* forty-five guinea pigs, divided into three groups of fifteen animals each (Groups II, III, and IV of Table II), were sensitized to picryl chloride by intraperitoneal injection, with and without the addition of the "procollagen adjuvant" or Freund's adjuvant, and challenged, four weeks after the first sensitizing injection, by the percutaneous and intracutaneous application of the allergen. Group I, consisting of fifteen nonsensitized controls, was kept untreated until the time of challenge. The animals in Group II were sensitized to picryl chloride alone, those of Group III were sensitized to picryl chloride combined with procollagen, and those of Group IV were

*I am grateful to my associate, Dr. H. Heymann, for the preparation of the procollagen and the adjuvant.

TABLE I. FIRST EXPERIMENT. SCHEDULE OF SENSITIZING INJECTIONS

DAYS	PREPARATIONS ADMINISTERED INTRAPERITONEALLY PER GUINEA PIG		
	GROUP II	GROUP III	GROUP IV
1	—	0.5 ml. procollagen preparation combined with 0.2 mg. picryl chloride	0.5 ml. Freund's adjuvant
2	—	—	—
3	0.2 mg. picryl chloride in 0.5 ml. saline	—	0.2 mg. picryl chloride in 0.5 ml. sodium chloride
4	—	—	—
5	—	0.5 ml. procollagen preparation combined with 0.2 mg. picryl chloride	0.5 ml. Freund's adjuvant
6	—	—	—
7	—	—	—
8	0.2 mg. picryl chloride in 0.5 ml. saline	—	0.2 mg. picryl chloride in 0.5 ml. saline

sensitized to picryl chloride combined with Freund's adjuvant. Thirty-seven animals were alive at the end of the four-week incubation period. The schedule of sensitizing injections is given in Table I, the results of the challenge in Tables II and III and Fig. 1.

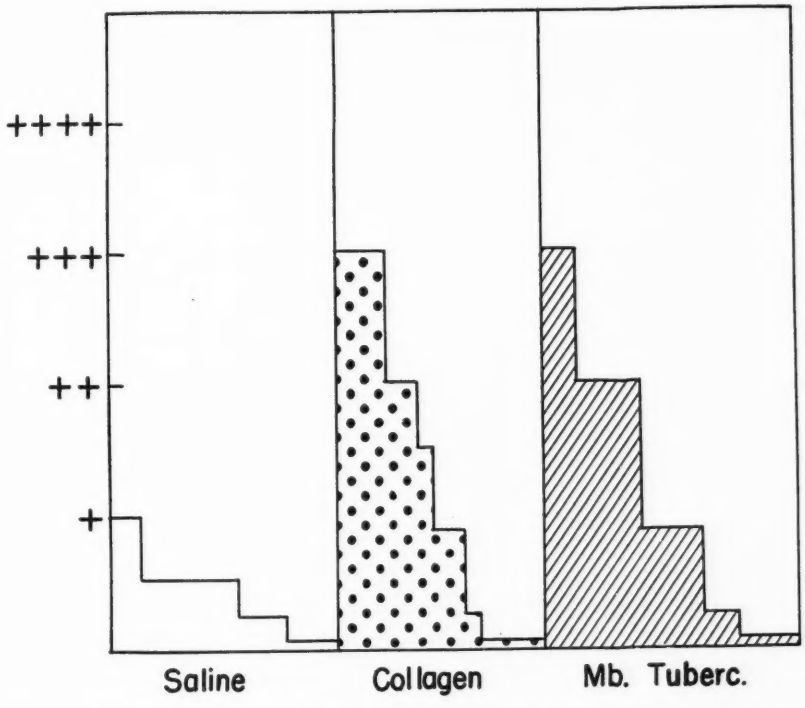

Fig. 1.—First experiment. Challenge reactions after sensitization to picryl chloride with saline, collagen adjuvant, and Mycobacterium tuberculosis (Freund's) adjuvant.

TABLE II. FIRST EXPERIMENT. RESULTS OF CHALLENGE APPLICATION

SENSITIZING TREATMENT	GUINEA PIG NO.	CHALLENGED BY SKIN SMEAR (24 HOUR READING)	CHALLENGED INTRADERMALLY (24 HOUR READING)
Group I. Normal, unsensitized controls	1	tr	0
	2	tr	0
	3	tr	0
	4	tr	0
	5	tr	0
	6	tr	0
	7	tr	0
	8	tr	0
	9	tr	0
	10	0	0
	11	0	0
	12	0	0
	13	0	0
	14	0	0
	15	0	0
Index		0	0
Group II. Picryl chloride in saline	16	++	+
	17	+	(+)
	18	(+)	(+)
	19	(+)	(+)
	20	(+)	0
	21	(+)	0
	22	(+)	0
	23	(+)	0
	24	tr	0
	25	tr	0
	26	tr	0
	27	0	0
	28	0	0
	29	0	0
Index		0.11	0.05
Group III. Picryl chloride with "procollagen adjuvant"	30	+++	+++
	31	++	++
	32	++	++
	33	++	++
	34	++	+ ½
	35	+ ½	+ ½
	36	+	+
	37	+	+
	38	(+)	+
	39	(+)	(+)
	40	tr	tr
	41	0	0
	42	0	0
	43	0	0
	44	0	0
Index		0.28	0.3
Group IV. Picryl chloride with Freund's adjuvant	45	+++	++
	46	++	+
	47	++	+
	48	+	0
	49	+	0
	50	tr	0
	51	0	0
	52	0	0
Index		0.32	0.16

In this and the following tables the intensities of reactions were recorded as 0, tr (trace), (+), +, + ½, ++, ++ ½, +++, +++ ½, and ++++. The index values for each group were calculated as follows: 0 and trace were considered as negative, the others were assigned numerical values of 1 to 8, respectively. The index for each group was obtained by dividing the total numerical value calculated for each group by the theoretical maximum value obtainable (number of animals per group × 8).

TABLE III. SUMMARY OF FIRST EXPERIMENT. RELATIVE INDEX VALUES

GROUP	TREATMENT	SMEAR CHALLENGE (24 HOUR READING)	INTRADERMAL CHALLENGE (24 HOUR READING)
I	Controls (unsensitized)	0.00	0.00
II	Picryl chloride only	0.11	0.05
III	Picryl chloride and procollagen	0.28	0.30
IV	Picryl chloride and Freund's adjuvant	0.32	0.16

Results.—The comparison of the challenge reactions observed twenty-four hours after tests with skin smear and by intradermal injection indicate that epidermal challenge usually produced a slightly higher number of generally weak positive reactions. In this experiment, preparation with picryl chloride in saline without addition of any adjuvant resulted in only two distinctly positive reactions (Group II). The addition of the procollagen adjuvant resulted in eight strongly positive reactivities (Group III). The addition of Freund's adjuvant was followed by a comparable increase of positive reactions (Group IV). As seen from Table III, the relative index values of the two groups (III and IV) receiving either adjuvant were three times as high as the index of Group II, which had received only picryl chloride.

2. In the *second experiment* the influence of a "procollagen adjuvant" and of an "albumin adjuvant" on the number of delayed sensitizations was investigated. Seventy-five guinea pigs, divided into four groups, were sensitized to picryl chloride alone or with addition of various adjuvants containing procollagen, bovine serum albumin, and Freund's adjuvant, respectively. The injection schedule is inscribed in Table IV, the individual results in Table V, and the index values in Table VI (Figs. 2 and 3).

TABLE IV. SECOND EXPERIMENT. SCHEDULE OF SENSITIZING INJECTIONS

DAY	PREPARATIONS ADMINISTERED INTRAPERITONEALLY PER GUINEA PIG			
	GROUP II	GROUP III	GROUP IV	GROUP V
1	—	0.5 ml. procollagen preparation combined with 0.2 mg. picryl chloride	0.5 ml. bovine serum albumin combined with 0.2 mg. picryl chloride	0.5 ml. Freund's adjuvant
2	—	—	—	—
3	0.2 mg. picryl chloride in 0.5 ml. saline	—	—	0.2 mg. picryl chloride in 0.5 ml. saline
4	—	—	—	—
5	—	0.5 ml. procollagen preparation combined with 0.2 mg. picryl chloride	0.5 ml. bovine serum albumin combined with 0.2 mg. picryl chloride	0.5 ml. Freund's adjuvant
6	—	—	—	—
7	—	—	—	—
8	0.2 mg. picryl chloride in 0.5 ml. saline	—	—	0.2 mg. picryl chloride in 0.5 ml. saline

Group I: untreated controls.

TABLE V. SECOND EXPERIMENT. RESULTS OF CHALLENGE APPLICATIONS

SENSITIZING TREATMENT	GUINEA PIG NO.	CHALLENGED BY SKIN SMEAR (24 HOUR READING)	CHALLENGED INTRADERMALLY (24 HOUR READING)
Group I. Normal, not sensitized controls	1	+	+
	2	(+)	(+)
	3	(+)	tr
	4	(+)	tr
	5	(+)	tr
	6	(+)	tr
	7	tr	tr
	8	tr	0
	9	tr	0
	10	tr	0
	11	tr	0
	12	0	0
	13	0	0
	14	0	0
Index		0.06	0.03
Group II. Picryl chloride in saline	15	++	++
	16	+	+
	17	+	+
	18	(+)	+
	19	(+)	(+)
	20	(+)	tr
	21	(+)	tr
	22	tr	0
	23	tr	0
	24	tr	0
	25	tr	0
	26	tr	0
	27	0	0
	28	0	0
Index		0.1	0.1
Group III. Picryl chloride with "procollagen adjuvant"	29	+++	+++
	30	+++	++
	31	++ ½	++
	32	++	+ ½
	33	++	+
	34	+ ½	+
	35	+ ½	+
	36	+	+
	37	+	+
	38	(+)	+
	39	tr	(+)
	40	tr	(+)
	41	tr	tr
	42	tr	tr
Index		0.3	0.3
Group IV. Picryl chloride and bovine serum albumin	43	+	+
	44	tr	(+)
	45	tr	(+)
	46	tr	tr
	47	tr	tr
	48	tr	0
	49	0	0
	50	0	0
	51	0	0
	52	0	0
	53	0	0
	54	0	0
Index		0.03	0.04

TABLE V—CONT'D

SENSITIZING TREATMENT	GUINEA PIG NO.	CHALLENGED BY SKIN SMEAR (24 HOUR READING)	CHALLENGED INTRADERMALLY (24 HOUR READING)
Group V. Picryl chloride and Freund's adjuvant	55	+++	+++
	56	+++	+++
	57	+++	++
	58	+++	++
	59	+++	++
	60	++	++
	61	++	++
	62	++	+ ½
	63	++	+
	64	++	+
	65	+ ½	+
	66	+ ½	+
	67	+	+
	68	+	(+)
	69	+	(+)
	70	+	(+)
	71	+	(+)
	72	(+)	(+)
	73	(+)	(+)
	74	(+)	(+)
	75	(+)	tr
	76	tr	tr
	77	tr	0
	78	tr	0
	79	0	0
	80	0	0
Index		0.34	0.25

Group I: untreated controls.

TABLE VI. SUMMARY OF SECOND EXPERIMENT. RELATIVE INDEX VALUES

GROUP	TREATMENT	SMEAR CHALLENGE (24 HOUR READING)	INTRADERMAL CHALLENGE (24 HOUR READING)
I	Controls	0.06	0.03
II	Picryl chloride only	0.1	0.1
III	Picryl chloride and procollagen	0.3	0.3
IV	Picryl chloride and serum albumin	0.03	0.04
V	Picryl chloride and Freund's adjuvant	0.34	0.25

Result.—While sensitizations with picryl chloride alone produced only a small number of positive reactions, with a relative index value of 0.1, the addition of procollagen gave rise to a considerable increase in number as well as intensity of positive reactions, with a threefold increase in the index value (0.3). The reactions obtained with Freund's adjuvant were very slightly stronger, having an index of 0.34 for the epidermal challenge, but slightly weaker with an index of 0.25 upon intradermal challenge. On the other hand, the addition of albumin resulted in a considerable decrease in reactivities with index values of only 0.03 to 0.04.

3. In the *third experiment* recorded, Old Tuberculin (O.T.) was used as sensitizing agent and the influence of procollagen adjuvant, albumin adjuvant,

and Freund's adjuvant upon the production of a sensitization of the delayed type was investigated. As in the previous experiment, the O.T., as well as the adjuvants, was injected intraperitoneally. In this experiment, seventy-five

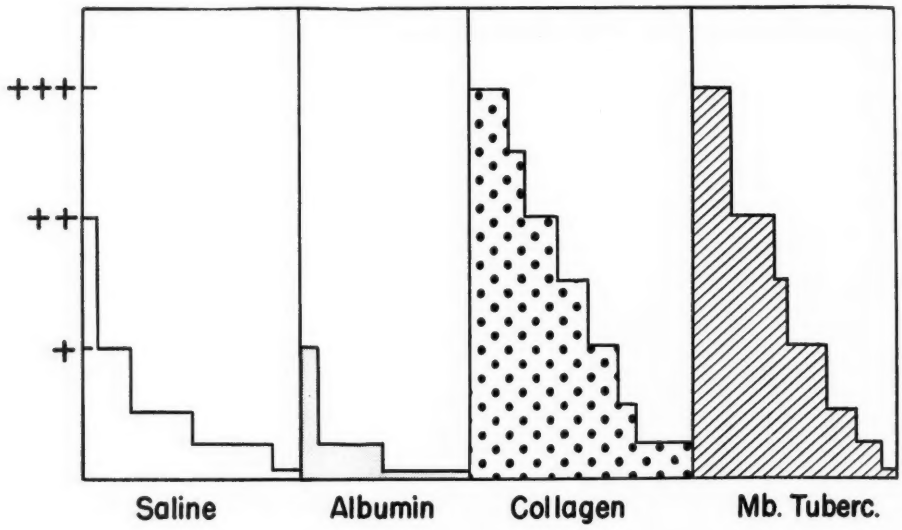

Fig. 2.—Second experiment. Challenge reactions after sensitization to picryl chloride with saline, albumin, collagen, and Mycobacterium tuberculosis (Freund's) adjuvant.

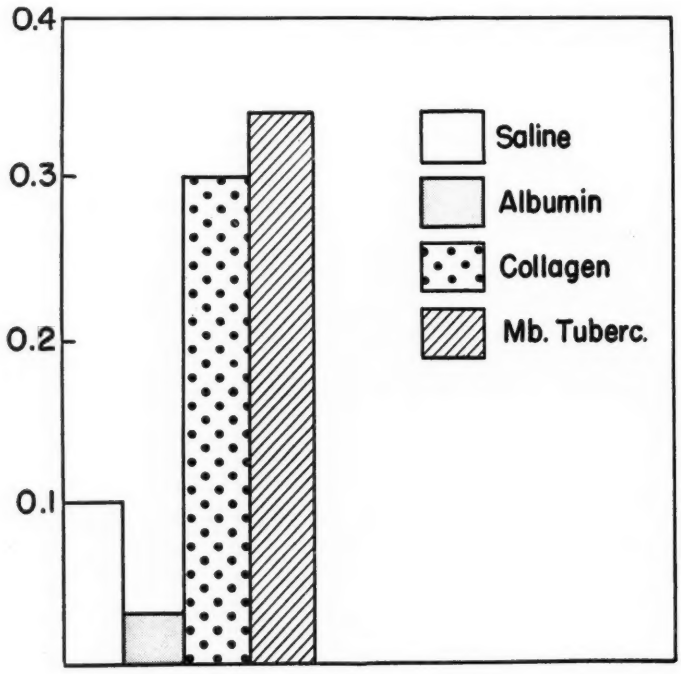

Fig 3.—Second experiment. Challenge reaction, indices.

TABLE VII. THIRD EXPERIMENT. SCHEDULE OF SENSITIZING INJECTIONS

DAYS	PREPARATIONS ADMINISTERED INTRAPERITONEALLY PER GUINEA PIG			
	GROUP II	GROUP III	GROUP IV	GROUP V
1	Nothing	0.5 ml.* procollagen combined with 0.1 ml. Old Tuberculin	0.5 ml. serum albumin combined with 0.1 ml. Old Tuberculin	0.5 ml. Freund's adjuvant
2	Nothing	Nothing	Nothing	Nothing
3	0.1 ml. Old Tuberculin in 0.5 ml. saline	Nothing	Nothing	0.1 ml. Old Tuberculin in 0.5 ml. saline
4	Nothing	Nothing	Nothing	Nothing
5	Nothing	0.5 ml.* procollagen combined with 0.1 ml. Old Tuberculin	0.5 ml. serum albumin combined with 0.1 ml. Old Tuberculin	0.5 ml. Freund's adjuvant
6	Nothing	Nothing	Nothing	Nothing
7	Nothing	Nothing	Nothing	Nothing
8	0.1 ml. Old Tuberculin in 0.5 ml. saline	Nothing	Nothing	0.1 ml. Old Tuberculin in 0.5 ml. saline

*The Old Tuberculin mixtures of procollagen and serum were used immediately upon preparation.

guinea pigs were sensitized to O.T. in groups of fifteen animals each. Seventy-one of the animals were alive at the moment of the challenge. The injection schedule of this experiment is inscribed in Table VII, the individual results in Table VIII, and the index values in Table IX and Fig. 4.

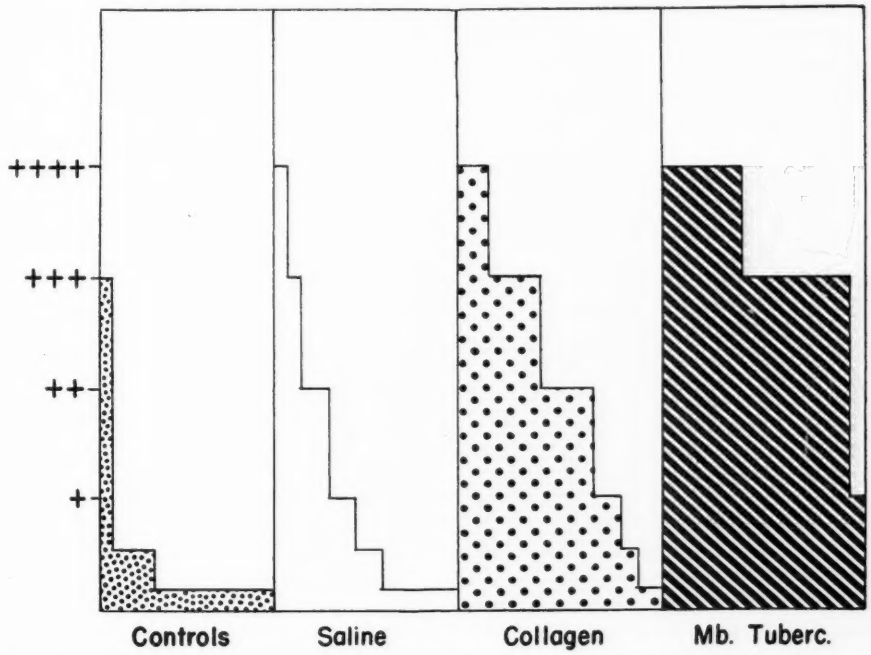

Fig. 4.—Third experiment. Challenge reactions after sensitization to Old Tuberculin with saline, collagen, and Mycobacterium tuberculosis (Freund's) adjuvant.

TABLE VIII. THIRD EXPERIMENT. RESULTS OF CHALLENGE APPLICATIONS

SENSITIZING TREATMENT	GUINEA PIG NO.	INTRADERMAL CHALLENGE 10%—24 HOURS
Group I. Normal controls (not sensitized)	1	+++
	2	(+)
	3	(+)
	4	(+)
	5	0
	6	0
	7	0
	8	0
	9	0
	10	0
	11	0
	12	0
	13	0
Index		0.09
Group II. Old Tuberculin in saline	14	++++
	15	+++
	16	++
	17	++
	18	+
	19	+
	20	(+)
	21	(+)
	22	0
	23	0
	24	0
	25	0
	26	0
	27	0
Index		0.25
Group III. Old Tuberculin and procollagen	28	++++
	29	++++
	30	+++
	31	+++
	32	+++
	33	+++
	34	++
	35	++
	36	++
	37	++
	38	+
	39	+
	40	(+)
	41	0
	42	0
Index		0.40
Group IV. Old Tuberculin and bovine serum albumin	43	+++
	44	+++
	45	+++
	46	++
	47	+
	48	+
	49	0
	50	0
	51	0
	52	0
	53	0
	54	0
Index		0.27

TABLE VIII.—CONT'D

SENSITIZING TREATMENT	GUINEA PIG NO.	INTRADERMAL CHALLENGE 10%—24 HOURS
Group V. Old Tuberculin and Freund's adjuvant	55	++++
	56	++++
	57	++++
	58	++++
	59	++++
	60	++++
	61	+++
	62	+++
	63	+++
	64	+++
	65	+++
	66	+++
	67	+++
	68	+++
	69	+
Index		0.81

TABLE IX. SUMMARY OF THIRD EXPERIMENT. RELATIVE INDEX VALUES

GROUP	TREATMENT	10%—24 HOURS
I	Controls	0.08
II	Old Tuberculin in saline	0.25
III	Procollagen and Old Tuberculin	0.40
IV	Bovine serum albumin and Old Tuberculin	0.27
V	Freund's adjuvant and Old Tuberculin	0.81

Results.—The results of this experiment indicate that a number of animals were tuberculin positive prior to the first O.T. injection of control Group I and the first challenge of the animals of Group II, since, under the conditions of this experiment, no sensitizations to O.T. could be expected unless an effective adjuvant was added. There was one strong positive reaction in the control group and four strong reactions in the animals of Group II treated with two injections of tuberculin alone and then held for four weeks without further treatment. Four positive reactions were also noted in Group IV, in which all animals received serum albumin in addition to O.T. The index values in these two groups were 0.25 and 0.27, respectively.

Despite this interference, the influence of the collagen adjuvant upon the successful sensitization was very noticeable. The percentage of strongly positive reactions in Group III (animals receiving procollagen) was almost twice that of the Group II guinea pigs receiving O.T. alone, with an index value for Group III of 0.40 as against 0.25 for Group II. It could be expected that the injection of Freund's adjuvant resulted in a strong increase of positive challenge reactions with an index of 0.8.

DISCUSSION

The results of the three experiments reported here indicate, in confirmation of our previous experiments, that in sensitizations of guinea pigs to picryl chloride procollagen exerts the same delayed-reaction-promoting effect as do tubercle bacilli or lipopolysaccharides isolated from the wax fraction of mycobacteria. Indeed, the challenge reactions of the delayed type were considerably stronger and more numerous when the sensitizations were carried out

with the addition of procollagen than in its absence. The activity of mycobacteria, however, in promoting delayed reactions upon sensitization with O.T. was markedly superior to that of procollagen.

While there can be little doubt that the hypersensitivities produced with picryl chloride represent allergies of the contact type, this is not a priori certain in the case of sensitizations obtained to O.T. The question arises whether the reactivities observed in the collagen-treated group are actually true delayed reactions of the tuberculin type and not immediate reactions of the Arthus type.

It was often observed that the positive skin reactions resulting from experimental sensitizations with O.T. alone faded more rapidly than those accompanying a tuberculous infection or following sensitization with dead mycobacteria. It has been questioned on this basis whether sensitizations after preparation with O.T. alone were indeed specific sensitizations to tuberculin and the skin reactions actual "tuberculin reactions." We have observed a similar, more rapid fading of delayed skin reactions in our experiments including those in which sensitizations to O.T. were obtained by the simultaneous use of the collagen adjuvant. On the other hand, the strongly positive challenge reactions persisted even after seventy-two hours in approximately 50 per cent of those animals which had been sensitized to O.T. with the aid of Freund's adjuvant; after seventy-two hours virtually all reactions induced by the use of procollagen and albumin, as well as those produced with O.T. alone, had faded. Nevertheless, it seems probable that these reactivities do not represent an Arthus type of immediate reaction, but types of true delayed reaction. It is known that in most instances the Arthus-type reaction reaches the maximum intensity after several hours and then slowly decreases to almost one-half this maximum after twenty-four hours, whereas the delayed type of reaction continues to increase in intensity up to twenty to twenty-four hours, when it reaches its maximum. In our case, maximum intensity was also reached after twenty-four hours. It seems, therefore, that the differences in the persistence of the skin reactions of sensitizations obtained in the presence of tubercle bacilli and of those obtained in association with collagen may be due to quantitative differences of the same (namely, tuberculin) type of reactivity, rather than to a different type. There is the possibility, however, that these reactions are delayed reactions in a hypersensitiveness not to the specific tuberculin protein, but to a nonspecific protein present in the Old Tuberculin. New experiments with purified protein fractions are under way.

We believe that these reported results represent additional support for our hypothesis that (1) the delayed reactivity is mediated by a complete antigen whose protein carrier is formed by a fibroid protein, such as collagen, while the immediate reactivity is mediated by a complete antigen whose protein carrier is a globular protein, such as albumin or globulin, and (2) that the delayed-reactivity-promoting effect of mycobacteria and associated waxes is due to the formation of tubercles and, within these tubercles, of collagen on sites normally poor in fibroid proteins.

SUMMARY

1. Procollagen, like mycobacteria, increases the delayed reactions following sensitization of guinea pigs with picryl chloride or with Old Tuberculin by the parenteral route. Albumin does not exert this effect.

2. The effect of tubercle bacilli and lipopolysaccharides isolated from the wax fraction of tubercle bacilli upon the production of sensitizations of the delayed type after intraperitoneal injection is explained by the formation of specific tubercles and consequent formation of fibroid protein, particularly collagen, at sites in the vicinity of the injected hapten, thus enabling the hapten to combine with collagen to form complete antigens containing a carrier of fibroid protein.

3. The theory is discussed that the sessile antibody of the delayed type of sensitization is formed by complete antigen with fibroid proteins as carrier, whereas the humoral antibodies of the immediate or anaphylactic type are produced by complete antigen with globular proteins as carriers.

REFERENCES

1. Landsteiner, K., and Chase, M. W.: J. Exper. Med. **66**: 337, 1937.
2. Chase, M. W.: J. Exper. Med. **86**: 489, 1947.
3. Landsteiner, K., and Chase, M. W.: J. Exper. Med. **71**: 237, 1940.
4. Raffel, S., Arnaud, L. E., Dukes, C. Dean, and Huang, J. S.: J. Exper. Med. **90**: 53, 1949.
5. Raffel, S.: Experientia **6**: 410, 1950.
6. Mayer, R. L.: Internat. Arch. Allergy **8**: 115, 1956.
7. Orekhovich, V. N., Turtanoviskii, A. A., Orekhovich, K. D., and Plotinkova, N. E.: C. A. **42**: 7805, 1948.
8. Rich, A. R.: The Pathogenesis of Tuberculosis, ed. 2, Springfield, Ill., 1951, Charles C Thomas, Publisher.

LEUKOCYTIC TRANSFER OF THE IMMEDIATE TYPES OF ALLERGIC REACTION IN MAN

I. Techniques for the Local Passive Transfer of Atopic Hypersensitiveness Through the Use of Leukocytes and Their Derivatives

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IN 1950, Walzer and Glazer[1] reported the passive local cutaneous sensitization of normal subjects by the intracutaneous injection of viable leukocytes obtained from atopic patients who manifested immediate whealing reactions to skin tests with common allergens. The method of Minor and Burnett,[2] slightly modified, was employed to separate the leukocytes from the donor's venous blood. By the addition of bovine fibrinogen, erythrocytic sedimentation was accelerated, leaving the white blood cells suspended in the plasma from which they were separated by centrifugation in Machlett tubes. The leukocytes were washed by centrifugation in changes of Tyrode's solution and were resuspended in fresh solution. Intracutaneous injections of this suspension in the arms of normal recipients provided passive transfer sites for subsequent tests with allergens.

Successful transfers of sensitivity to common allergens, such as ragweed pollen and rabbit dander, were demonstrated with the leukocytes of all but one of six allergic patients with positive skin reactions of average intensity to these allergens.[1] The immediate whealing reaction elicited by the allergen test at the passively sensitized site did not exceed a slight or a one-plus (+) positive. Nevertheless, these small reactions were elicited regularly in certain cases and were proved to be specific. The erythrocytes of the donor lacked the skin-sensitizing properties of his leukocytes.

The paramount difficulty encountered with this technique was the inflammatory reaction which developed in the recipient at the site of the sensitizing injection. This consisted of a central edematous area with superimposed and surrounding erythema. The inflammation was usually late in starting and developed slowly. It did not pass its peak until the fourth or fifth day, so that testing was rarely possible before the sixth to the eleventh day. Even at this time, traces of edema occasionally were still palpable, a circumstance which reduced the responsiveness of the site to the test antigen and made the reading and evaluation of the results more difficult. Not infrequently, the persistence of inflammation precluded any testing of the site.

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Among other difficulties encountered with the leukocytic suspension technique was the fact that the amount of inflammation which developed at the sensitized site varied with the recipient. In addition, attempts to enhance the sensitivity of passive transfer sites by concentrating the leukocytic suspension or by injecting larger amounts of it led to even greater local inflammation.

In the effort to circumvent some of these difficulties, many modifications of the suspension technique were tried. Early in the course of these studies (1951), it became apparent that viable leukocytes were not essential for a successful transfer. Positive results could be obtained with fragmented cells and even with extracts of crushed white blood cells. This finding became more significant when it was noted that the intracutaneous injection of such extracts produced little or no inflammatory reaction. This led to the development of a leukocytic extract technique for passive transfer testing which proved to be quite effective.

With the extract technique, the results previously obtained with the suspension method were confirmed. In many respects, the extract technique was found to present definite advantages over the suspension method, but it also proved to be subject to certain limitations. It could not, for example, replace the suspension technique in studies which required the transfer of normal, functioning leukocytes. It therefore became necessary to develop both techniques simultaneously. The significant findings uncovered during the course of these studies will be presented in this and subsequent articles.

No attempt will be made at this time to review the studies on the cellular transfer of the delayed types of allergic reactions which were originally reported by Landsteiner and Chase[7] and subsequently developed by many others. The immunologic mechanisms involved in the immediate and delayed types of allergic reaction are quite different. The same may be said of the diagnostic procedures which are used for the detection and differentiation of these two groups of hypersensitiveness and for the experimental techniques employed for their passive transfer. Discussions on passive transfer studies in delayed hypersensitiveness will therefore be deferred and will be dealt with in a future report.

MATERIALS AND METHODS

The Preparation of the Sensitizing Leukocytic Suspensions and Extracts.— Under sterile precautions which were maintained throughout the procedures, from 40 to 60 ml. of venous blood were drawn from the patient into tubes containing heparin in the proportion of 0.4 mg. for each 10 ml. of blood. After mixing gently by twice inverting the tubes, 1 ml. of a freshly prepared Seitz-filtered solution containing 40 mg. of bovine fibrinogen (Fraction I, Armour) was added for each 10 ml. of heparinized blood. The tubes were again inverted twice and the blood was transferred in 10 ml. aliquots to conical centrifuge tubes. Standing at an angle of 60 degrees, these were incubated at 37° C. for about one hour. The supernates were then transferred by capillary

pipette to Machlett capillary-tip tubes and centrifuged at 3,000 r.p.m. for approximately one hour. The supernate was removed and the sedimented leukocytes were resuspended in either 2 ml. of Tyrode's solution or 2 ml. of the recipient's freshly prepared blood serum. This suspension was centrifuged for thirty minutes at gradually increasing speeds up to 3,000 r.p.m. The washing procedure was then repeated with fresh Tyrode's solution or with serum. The sedimented leukocytes obtained at this point were ready for use in either the suspension or the extract technique.

For the *suspension technique,* one part cell sediment was distributed as evenly as possible in nine parts Tyrode's solution. This 1:10 suspension was then drawn up into a sterile syringe and used for the preparation of passive transfer sites on the arms of a recipient.

For the leukocytic *extraction technique,* one part cell sediment and three parts Tyrode's solution were introduced under sterile precautions into a small flat-bottomed vial. Three large sterile beads were added. The vial was sealed with a rubber stopper and was shaken overnight, usually for about sixteen hours, in a rotary machine at a speed of 300 r.p.m. The material in the vial was then transferred to a centrifuge tube and spun at 4,000 r.p.m. for twenty minutes. The clear supernate was drawn up into a sterile syringe and used for the preparation of passive transfer sites on the recipient.

An average yield of 0.028 ml. of sedimented cells was obtained in the earlier studies from each 10 ml. of donor's blood. In later studies, the more effective elimination of erythrocytes reduced the yield to approximately 0.02 ml. from each 10 ml. of blood. More than 85 per cent of the cells in this sediment were viable, as determined by their failure to take a trypan blue stain.

Variations in technique: In the attempt to achieve better transfers, many modifications of the techniques were tried. For washing the leukocytes, human nonreaginic serum was substituted for Tyrode's solution. The serum was most frequently obtained from the recipient on the day of the experiment. When two recipients participated in the same experiment, the serum of one was used for both. Human serum proved to be advantageous for the washing of the leukocytes only in those experiments in which the preservation of the cells was an important consideration. Otherwise, it produced results no more satisfactory than those obtained with Tyrode's solution. Washing the cells in Tyrode's solution containing 0.13 per cent gelatin provided leukocytes in a better state of preservation but did not improve the results of passive tranfer.

As a menstruum for the sensitizing leukocytic suspension, human serum proved inferior to Tyrode's solution. Serum tended to increase the hyporesponsiveness of the sensitized sites.[3] Moreover, its occasional use in recipients other than the one who provided the serum increased the possibility of introducing unrecognized immunologic factors at the sensitized sites.

Washing the cells by slow centrifugation four times instead of twice slightly reduced the size of the transfer reaction in some instances,[1] but not in others.

In the studies on the influence of time and temperature factors in the techniques, leukocytes and their derivatives were stored for twenty-four, forty-eight, and occasionally for seventy-two hours in the refrigerator with little perceptible effect on their sensitizing properties. Similarly, standing at room temperature for periods up to twenty-four hours did not noticeably influence the sensitizing properties of sterile leukocytic preparations. On the other hand, leukocytic suspensions stored in the incubator for more than a few hours produced marked delayed inflammatory reactions which rendered the passively sensitized sites unfit for testing.

The fact that the sensitizing principles of the leukocytic preparations were not affected by refrigerator or room temperatures during the first twenty-four-hour period eliminated the need for haste in the extraction of white blood cells. This proved fortunate, since the procedures which are commonly recommended for rapid fragmentation of leukocytes were not found to be applicable in these studies. For example, the triple alternate freezing (–70° C. in a dry ice and alcohol bath) and thawing (37° C.) of the leukocytes yielded, on centrifugation, a slightly hemorrhagic supernate which caused considerable inflammation and discoloration of the injected sites in the recipient. Other common methods of demolishing the leukocytes also proved impractical in these studies because of the small amounts of cells which were being employed.

Extraction of the white blood cells by shaking could be completed in less than sixteen hours. At times, it was possible to obtain positive results after only eight or twelve hours of shaking, even though the cells had not yet been completely demolished. Nevertheless, an overnight shaking period of sixteen hours proved both convenient and effective, since it was impossible to complete both the procurement and extraction of the leukocytes in a single working day. The extended shaking period not only assured complete demolition of the cells, but also permitted the performance of the active phases of the procedure during routine laboratory working hours.

*Passive Local Sensitization of the Recipients.**—

1. *Choice of recipients:* These were carefully selected nonatopic young adults with normal skins who, on preliminary testing, had shown completely negative reactions to concentrated extracts of the allergens employed in these studies as well as to a representative series of common allergenic offenders. Some recipients with particularly suitable skins were used repeatedly at appropriate intervals. This proved helpful in studying the effects of modifications in the technical procedures. It is noteworthy that the repeated injections of viable cells from the same or different sensitive donors produced no evidence of the development of generalized cutaneous sensitivity in the recipient.

2. *The preparation of cutaneous sites on the recipient:* In the earliest studies,[1] passive transfer sites were prepared by the intracutaneous injection of 0.05 ml. of a 1:13 suspension of leukocytes. Many of these sites developed

*For detailed description and discussion of the techniques employed in routine (indirect testing) and special passive transfer procedures, the reader is referred to previous discussions on this subject by Walzer and Bowman.[4]

marked inflammation and were unfit for testing. This difficulty was partially overcome by reducing the volume of the sensitizing injection to 0.025 ml. and increasing the concentration of the leukocytic suspension from 1:13 to 1:10. With this smaller sensitizing dose, the sites recovered their normal appearance and responsiveness much more rapidly.

Because the leukocytic extract failed to produce the pronounced inflammatory response observed with the cell suspension, the injection of 0.05 ml. of extract into each site proved feasible. Sites were sensitized on the outer aspects of the recipient's arms, not more than four sites being prepared on each arm. The marking of the sites and instructions to the recipient concerning the preservation of the markings and the avoidance of particular allergenic contacts were the same as those employed in routine passive transfer procedures.[4]

The Testing of the Sites.—

1. *Interval:* The inflammatory reactions which developed at the sites of injection of leukocytic suspensions prevented skin testing before the sixth day, and frequently further postponement was necessary. If the sites failed to regain their normal appearance by the fourteenth day, no testing was done. Because the inflammatory factor was a minor consideration with the extract technique, sites prepared in this manner were usually suitable for testing within a few days. However, in keeping with the principles recommended for all passive transfer procedures,[4] testing was rarely performed before the fifth day. This interval allowed the sensitized sites to recover most of the responsiveness which had been impaired by the trauma of the sensitizing injection.[3]

2. *Allergens:* The allergens employed for testing were those prepared in the Allergy Laboratory of the Jewish Hospital of Brooklyn for routine use in the Allergy Clinic.[5] They were standardized on the basis of their total nitrogen content. The ragweed pollen extracts were made annually in January from mixtures of giant and dwarf ragweed. Because of the tendency for these extracts to lose some of their potency with aging, fresh pollen extracts (labeled SP in the tables) were often prepared and used instead of, or in addition to, the stock extracts. These special (SP) ragweed extracts were usually prepared from dwarf ragweed pollen only.

3. *Testing:* About 0.02 ml. of allergenic extract was used for testing a passive transfer site. The allergen concentration was the highest which could be employed without producing a nonspecific positive reaction on the *normal* skin of the recipient. This concentration naturally varied with the allergen and the nature of the recipient's skin. With pollens, a solution containing 0.1 mg. nitrogen per milliliter proved to be suitable for most recipients. Occasionally tests were made with extracts containing 0.05 or 0.2 mg. of nitrogen per milliliter but the results obtained with these concentrations did not differ materially from those produced by the 0.1 mg. tests.

4. *Controls:* A control test, an exact duplicate of that made on the sensitized site, was made on the adjacent normal skin of the recipient. If enough

suitable transfer sites were available, one site was usually tested with diluting fluid and another with a suitable concentration of an unrelated antigen, that is, a member of a biologic group different from the one under consideration and one to which the donor failed to react either clinically or by skin test. In each instance, the tests were duplicated on adjacent normal skin.

In some of the earlier experiments, additional control tests were made on transfer sites prepared with leukocytic preparations obtained from nonsensitive donors. In other studies, sites prepared with erythrocytes from sensitive donors were tested. Since these special types of control tests uniformly yielded negative results, they were eventually discontinued.

The Reactions at the Transfer Sites.—The positive reactions elicited by tests with allergens at leukocytic transfer sites have always been small and have rarely exceeded the equivalent of a slight or one-plus reaction. With the extract technique, these reactions usually reached their maximum in about fifteen to twenty minutes and closely resembled those elicited in routine passive transfer testing. They consisted primarily of a central edema or wheal and variable amounts of erythema. Because of the small size of the reactions, the erythema was frequently faint and limited to the central edematous area. In the larger reactions, a secondary areola of solid or broken erythema was usually present. With the suspension technique, the reactions tended to develop somewhat more slowly and to show smaller areolas than those elicited with the extract technique. Pruritus infrequently accompanied positive reactions and, when present, was always minimal.

The Reading and Recording of Reactions.—Because slight inflammation frequently persisted at the sensitized sites, especially at those prepared with leukocytic suspensions, readings were routinely made on the transfer sites by both inspection and palpation *before* the allergens were introduced. Readings were taken again about twenty minutes after testing at both test and control sites. The amounts of edema and erythema were read and recorded separately. Pruritus was also noted when it occurred. Because edema tends to be absorbed more slowly in specific reactions than in nonspecific cutaneous responses, the reactions to the tests were frequently studied for periods up to an hour.

Grading of Reactions.—Because the small reactions obtained with the leukocytic transfer technique were more significant than similar responses in other passive transfer techniques, it became necessary to devise a new and more precise scale for the grading of weak positive reactions. For this purpose, reactions between the completely negative (0) response and the definite slight positive (+) response were divided into two grades, negative plus-minus (0±) and plus-minus positive (±). A description of the various grades of reactions, their symbols, readings, and significance, appear in Table I.

The slight positive or one-plus (+) reaction consists of a central wheal about 0.5 cm. in diameter with a surrounding erythema and is only occasionally accompanied by a faint pruritus. Reactions slightly less than this were

called plus-minus (±) positives. Erythema was not regularly present in this grade of reaction, and pruritus was rare. When the presence of edema was barely perceptible, the reaction was considered to be negative plus-minus (0±). A surrounding erythema was usually absent in this grade of reaction. When no change in edema or erythema was observed following the test, the reaction was recorded as negative (0).

TABLE I. THE GRADING OF IMMEDIATE PASSIVE TRANSFER REACTIONS OBTAINED WITH THE LEUKOCYTIC TECHNIQUES

READING	SYMBOL	DESCRIPTION	INTERPRETATION
Negative	0	Site unchanged	Negative
Negative—plus-minus	0±	Barely perceptible edema	Too weak to be regarded as significant
Plus-minus positive	±	Definite but small amount of edema, often with faint erythema on or around it	A weak positive reaction which is significant if it is adequately controlled and can be confirmed on repetition of the test on another site
Slight positive	+	Wheal at least 0.5 cm. in diameter with definite areola	A definite and significant positive transfer reaction

Evaluation of Results.—The evaluation of results of the passive transfer tests was based upon the comparison of the reactions at the sensitized sites with those elicited by the various control tests. Edema or wheal formation represented the primary basis of comparison. Erythema and pruritus, when present, were recognized as additional but secondary and less reliable criteria of positive reactions.

The evaluation of results with the extract technique differed very little from the procedure employed in routine passive transfer tests[4] and presented no unusual difficulties. With the suspension technique, however, evaluation was more complicated. The reading at a sensitized site was accepted without correction only when the site was completely normal at the time of testing and all the controls were completely negative. Otherwise the amount of site edema which preceded testing or which developed at a control site had to be subtracted from the reading on the sensitized site. All readings in the text and tables represent the final results after all corrections have been made.

Interpretation of Results.—In the interpretation of results, the slight positive (+) reaction was considered to be definitely significant. A plus-minus positive (±) was deemed to be somewhat less reliable than the slight positive (+), but was considered to be valid when it could be confirmed by repetition on fresh sites and when its controls were adequate. Since the negative plus-minus (0±) reaction might well be the result of technical variation, it was not considered to be significant in itself and was usually grouped with the negative reactions.

PASSIVE TRANSFER EXPERIMENTS WITH LEUKOCYTES FROM DONOR D. G.

Most of the donors studied were atopic patients with immediate positive whealing reactions to tests with common allergens. Not all these reactions

were clinically significant. Atopic reagins in low or average titer were present in these patients' sera. Some cases illustrating nonatopic forms of hypersensitiveness in which immediate whealing reactions occur were also tested. These studies will be the subject of a subsequent report.

In studying the effects of variations in technique, we found that it was advantageous to use the same donor and recipient repeatedly. For this reason, D. G., an atopic young male adult who, since infancy had been under the observation of one of the authors, was selected to provide the leukocytes for many of these studies. Only a few relevant details of his history are presented here, since a complete report of this case will appear in another communication.

Clinical History of D. G.—This 24-year-old man has a strong family history of atopy. As an infant, he had eczema for several months. Since then he has at various times manifested symptoms of allergic rhinitis, asthma, urticaria, and alimentary disturbances. Skin testing by the indirect method in childhood and by the direct intracutaneous technique in subsequent years revealed many positive reactions to foods and inhalants. In recent years, hay fever has been the predominating allergic symptom and practically all the leukocytic transfer studies on this patient were done in relation to his pollen allergy.

D. G. developed ragweed hay fever symptoms at the age of 12 (1944). Preseasonal treatment with ragweed was started and continued on a perennial basis, doses being given at monthly intervals. For many years the skin reaction to timothy pollen was strongly positive, but no clinical sensitivity to grass pollens developed. At the age of 21 (1953), the patient's serum showed moderate reaginic titers to both pollens by the method of Coca and Grove.[6] Serum dilution 1:64 gave a one-plus (+) transfer reaction to tests with ragweed and timothy pollen extracts containing 0.01 mg. nitrogen per milliliter. With serum dilution 1:256 and the same test extracts, plus-minus (±) reactions were obtained to both pollens, although the response to timothy was slightly weaker than that to ragweed.

Passive Transfer Studies With Suspensions of Leukocytes of Donor D. G.—From November, 1950, to April, 1951, the successful transfer of ragweed sensitivity was repeatedly demonstrated with suspensions of leukocytes of donor D. G. Some representative examples of the results obtained in those studies are listed in Table II. In most experiments, the leukocytes were washed and suspended in Tyrode's solution. The sensitizing injection, in the early trials, consisted of 0.05 ml. of a 1:10 leukocytic suspension but in later studies the sensitizing dose was reduced to 0.025 ml. for reasons previously noted. (See Materials and Methods.) Several recipients were used, most of them repeatedly (Tables II, III, and IV). The interval between the preparation and testing of sites usually lasted from six to eleven days. Stock or freshly prepared (SP) timothy and ragweed pollen extracts containing 0.1 mg. nitrogen per milliliter were used for testing.

1. *Passive transfer tests with ragweed pollen extracts:* With the leukocytic suspension technique, positive passive transfer reactions to ragweed

pollen extracts were obtained fairly regularly with the cells of D. G., although the degree of reactivity varied with the recipient. Occasionally there was some variation in response at different sites on the same recipient, which could be attributed to the difficulty in obtaining a uniform suspension of the leukocytes. The largest transfers to ragweed were one-plus (+) reactions, but weaker positives (±) were quite common. Negative (0) or negligible (0±) reactions to ragweed were infrequent with this donor's cells when the standard technique and suitable recipients were employed.

TABLE II. PASSIVE TRANSFER EXPERIMENTS WITH LEUKOCYTIC SUSPENSIONS OF DONOR D. G.

EXPERIMENT NO.	DATE OF BLEEDING AND SENSITIZATION	SENSITIZATION OF SITE		INTERVAL (DAYS)	TESTING OF SITE		INTERPRETATION OF RESULTS
		RECIPIENT	AMOUNT INJECTED (ML.)		ALLERGEN	REACTION OBTAINED‡	
S 1a	11/ 9/50	SC	0.05	11	Ragweed SP*	+	Positive
					Timothy SP	0	Negative
S 1b		G	0.05	7	Ragweed 50†	±	Weak positive
					Ragweed SP	+	Positive
					Timothy SP	0±	Negative
					Diluting fluid	0±	Negative
S 2a	2/13/51	G	0.05	6	Ragweed 51	+	Positive
		G				±	Weak positive
						+	Positive
S 2b		G	0.05	5	Ragweed 51	±	Weak positive
S 3	3/ 6/51	G	0.025	7	Ragweed 51	+	Positive
			0.05			±	Weak positive
S 4a	3/20/51		0.025	10	Ragweed 51	±	Weak positive
S 4c	3/24/51	G	0.025	6	Ragweed 51	+	Positive

*SP = Recently prepared extract.

†Ragweed 50 = Stock extract of ragweed pollen prepared in January, 1950.

‡Corrected readings:

Sites tested intracutaneously with 0.02 ml. of pollen extract containing 0.1 mg. introgen per milliliter.

Leukocytes washed with Tyrode's solution.

Sensitizing suspensions made of one part leukocytic sediment and nine parts Tyrode's solution.

2. *Passive transfer tests with timothy pollen extracts:* Although the reaginic titers for timothy and ragweed pollens were virtually equal in the serum of D. G., positive passive transfers to timothy were never elicited with his leukocytes by any technique. As the studies progressed, it became apparent that this failure did not rest on any inadequacies of method, but on some immunologic consideration. The fact that D. G. had no clinical symptoms of timothy hay fever, despite his strongly positive skin reaction and high reaginic titer for this pollen, prompted a more intensive investigation into the relationship between leukocytic transfer and clinical symptoms. These studies will be described in detail in a subsequent communication.

Passive Transfer Studies With Extracts of Leukocytes of Donor D. G.— When the passive transfer of positive skin reactions by means of extracts of leukocytes was successfully accomplished in April, 1951, studies on donor D. G. were continued with emphasis on this new technique. Representative

examples of the results obtained with the extract technique in this case are presented in Table III. Tyrode's solution or the serum of the recipient was used for the washing and extraction of leukocytes. In the earlier trials, one part cell sediment was shaken in five parts diluent (recorded as a 1:6 sensitizing extract in the tables). In later studies, a more concentrated extract (1:4) was successfully employed. Of this, 0.05 ml. was injected at each cutaneous site. The interval between site preparation and testing was usually from five to nine days.

TABLE III. PASSIVE TRANSFER EXPERIMENTS WITH LEUKOCYTIC EXTRACTS OF DONOR D. G.

EXPERIMENT NO.	DATE OF BLEEDING	PREPARATION OF LEUKOCYTIC EXTRACT: CELLS WASHED AND EXTRACTED WITH*	PREPARATION OF LEUKOCYTIC EXTRACT: CELL EXTRACT†	SENSITIZATION OF SITE: DATE	SENSITIZATION OF SITE: RECIPIENT	INTERVAL (DAYS)	TESTING OF SITE: ALLERGEN (MG. N/ML.)	TESTING OF SITE: REACTION OBTAINED	INTERPRETATION OF RESULTS
E 5a	4/10/51	T	1:6	4/11/51	S	7	Ragweed 51 0.1	+	Positive
E 5b				4/11/51	W	9	Ragweed 51 0.1	+	Positive
							Timothy 51 0.1	0±	Negative
							Peanut 0.05	0	Negative
E 15a	3/26/52	S	1:6	3/27/52	F	5	Ragweed 52 0.1	+	Positive
								±	Weak positive
							Timothy 52 0.1	0	Negative
E 15b				3/27/52	W	8	Ragweed 52 0.1	+	Positive
E 17	4/ 7/52	S	1:6	4/ 8/52	G	7	Ragweed 52 0.1	+	Positive
							Ragweed SP 0.1	+	Positive
							Timothy 52 0.1	0	Negative
E 19	4/16/52	S	1:6	4/17/52	S	7	Ragweed SP 0.1	+	Positive
							Ragweed 52 0.1	±	Weak positive
							Timothy 52 0.1	0	Negative
E 20	4/21/52	S	1:6	4/22/52	G	6	Ragweed 52 0.1	±	Weak positive
							Ragweed SP 0.1	+	Positive
							Timothy 52 0.1	0	Negative
E 28	5/27/52	S	1:4	5/28/52	G	8	Ragweed SP 0.1	+	Positive
							Ragweed 52 0.1	±	Weak positive
E 35	4/ 8/53	T	1:4	4/ 9/53	K	8	Ragweed 53 0.1	+	Positive
							Timothy 53 0.1	0	Negative
E 37	4/29/53	T	1:4	4/30/53	K	7	Ragweed 53 0.1	+	Positive
							Timothy 53 0.1	0	Negative

*T = Tyrode's solution; S = serum of recipient; SP = recently prepared extract.

†Sensitizing extract prepared by shaking one part cell sediment in three or five parts diluent to form approximately a 1:4 or 1:6 extract. Average shaking time, sixteen hours. Amount injected into each site, 0.05 ml.

As with the cell suspension technique, positive transfer reactions to ragweed were regularly obtained with the extract method. These reactions varied from + to ± in intensity, with less tendency to disparity than had been

TABLE IV. COMPARATIVE PASSIVE TRANSFER TESTS WITH THE TWO TECHNIQUES USING IDENTICAL BLOOD SAMPLES OF DONOR D. G.

EXPERIMENT NO.	DATE OF BLEEDING	CELL PREPARATION			SENSITIZATION OF SITES		INTERVAL (DAYS)	TESTING OF SITE		INTERPRETATION OF RESULTS
		CELLS WASHED WITH*	SUSPENSION (DILUENT AND DILUTION)	EXTRACT (DILUENT AND DILUTION)†	DATE	AMOUNT INJECTED (ML.)		ALLERGEN (MG. N/ML.)	REACTION OBTAINED	
S 7	5/1/51	T	T 1:10		5/ 2/51	0.025	7	R51 0.2	+	Positive
E 7	5/1/51			T 1:6	5/ 2/51	0.05	7	R51 0.2	+	Positive
S 29	6/9/52	S	S 1:10		6/ 9/52	0.025	11	R52 0.1	+	Positive
								RSP 0.1	+	Positive
		S	S 1:10					RSP 0.1	+	Positive
		S	S 1:10					RSP 0.2	±	Weak positive
E 29	6/9/52	S		S 1:4	6/10/52	0.05	9	R52 0.1	±	Weak positive
								RSP 0.1	+	Positive
								RSP 0.2	+	Positive

*T = Tyrode's solution; S = serum of recipient; SP = recently prepared extract; R = ragweed pollen extract.
†One part cell sediment shaken in three or five parts diluent to form approximately a 1:4 or 1:6 extract.
All tests performed on one recipient, S. W.

observed with the suspension technique. As had been the case in studies using the suspension technique, passive transfer reactions with timothy pollen were also consistently negative when the extract technique was employed.

Comparison of Results Obtained With Both Techniques Using a Single Blood Sample.—In the experiments recorded in Tables II and III, the blood samples of D. G. were taken on different days and processed by either the suspension or extract technique with frequent variations in both procedures. In only a few instances was it feasible to run both techniques simultaneously on aliquots of the same blood sample. The results of such comparative studies on cells of D. G. are presented in Table IV. These comparisons revealed no distinct superiority of either technique in demonstrating the transfer of ragweed sensitivity in the case of D. G.

A COMPARISON OF THE TWO TECHNIQUES

Comparative tests on specimens obtained from other donors, which are being reported elsewhere, tended to confirm the impressions gained from the

TABLE V. A COMPARISON OF THE LEUKOCYTIC SUSPENSION AND EXTRACT TECHNIQUES

	SUSPENSION TECHNIQUE	EXTRACT TECHNIQUE
1. The nature of the transferred material	A suspension of viable leukocytes	The extracted products of shattered leukocytes
2. Time required for processing transferred material	Approximately 8 hours	At least 24 hours
3. The concentration of the transferred material	1 part leukocytic sediment suspended in 9 parts of diluent (1:10)	One part leukocytic sediment shaken in 3 parts diluent (1:4)
4. The amount injected at each cutaneous site	0.025 ml.	0.05 ml.
5. The amount of inflammation developing at the site of injection	Varies from little to much	Varies from none to little
6. The variability in site inflammation:		
(a) in different recipients	Considerable	Slight
(b) at different sites on same recipient	Slight	Negligible
7. The average interval between preparation and testing of sites	From 6 to 11 days	From 5 to 9 days
8. The reading and interpretation of test results	Rendered difficult by the preceding site inflammation	Presents no unusual problem
9. The maximal passive transfer reaction obtained	One plus (+)	One plus (+)

studies on D. G. that the two methods seemed equally effective for the localized transfer of the atopic type of sensitivity. As the work progressed, various features developed in connection with both techniques which served to limit their usefulness in certain types of experiments. The most important of these considerations are summarized briefly and tabulated in Table V.

From a comparative analysis of the two techniques, it becomes obvious that the suspension method is irreplaceable where the studies require the transfer of viable cells. Moreover, the preparation of sensitizing materials in the suspension method is a shorter procedure. In most other respects, however, the extract technique seems to have more advantages. Its greatest asset is that it provides a passive transfer test site which, from its inception, usually passes through no pronounced inflammatory phase and at the time of testing tends to be relatively normal in its appearance and responsiveness. These site qualities are especially important in a passive transfer procedure in which the maximal reaction obtained does not exceed a one-plus (+) positive. The fact that sites prepared with the extract technique can be tested and read in practically the same manner as are serum-sensitized sites in routine indirect testing[4] will unquestionably appeal to the technician or clinician who is already well versed in passive transfer procedures.

It is advisable, nevertheless, to qualify these early impressions on the comparative merits of the two procedures by the consideration that so many of the studies which prompted them were made on the cells from a single donor. Tests with the cells of other atopic donors have tended to support these preliminary impressions. However, the total number of patients studied, the diversity of atopic manifestations, and the types of allergens investigated are still relatively limited. Moreover, the allergic mechanisms which are characterized by immediate positive skin reactions and may be regularly transferred by means of leukocytes are still to be defined. Furthermore, the evidence is by no means complete that the leukocytic extract will transfer *every* sensitizing principle which may be carried by viable leukocytes. For these reasons, a much larger and more diversified series of comparative studies will be needed before final conclusions can be reached as to the comparative values of the two techniques reported here.

SUMMARY

The local passive transfer of the atopic wheal type of skin reaction, which had previously been effected with suspensions of viable leukocytes, has now been accomplished by the use of extracts of fragmented white blood cells. The suspension and extraction techniques have been described in detail. They have thus far been found to be equally successful for passive transfer except in those experiments in which the preservation of cell viability is a prerequisite, in which case the suspension technique is obligatory. The relative merits and disadvantages of both procedures have been reviewed. The absence of a pronounced inflammatory response at passive transfer sites prepared with leukocytic extracts minimizes most of the difficulties encountered in the testing and reading of sites prepared with leukocytic suspensions.

REFERENCES

1. Walzer, M., and Glazer, I.: Passive Transfer of Atopic Hypersensitiveness in Man by Means of Leucocytes, Proc. Soc. Exper. Biol. & Med. **74**: 872, 1950.

2. Minor, A. H., and Burnett, L.: Method for Obtaining Living Leucocytes From Human Peripheral Blood by Acceleration of Erythrocyte Sedimentation, Blood **3**: 799, 1948.
3. Bowman, K. L., and Walzer, M.: Studies in Reaginic and Histaminic Wheals. I. The Effects of Reaginic and Histaminic Wheals Upon the Subsequent Responsiveness of Passively Sensitized Cutaneous Sites, J. ALLERGY **24**: 126, 1953.
4. Walzer, M., and Bowman, K. L.: The Indirect Method of Testing, in Coca, A. F., Walzer, M., and Thommen, A. A.: Asthma and Hay Fever in Theory and Practice, Springfield, Ill., 1931, Charles C Thomas, Publisher, p. 356.
Walzer, M., and Bowman, K. L.: The Indirect Method of Testing, in Cooke, R. A.: Allergy in Theory and Practice, Philadelphia, 1947, W. B. Saunders Company, p. 507.
5. Coca, A. F., and Milford, E. L.: Studies in Hypersensitiveness. XVII. The Preparation of Fluid Extracts and Solutions for Use in the Diagnosis and Treatment of Atopic Conditions, J. Immunol. **10**: 555, 1925.
6. Coca, A. F., and Grove, E. F.: Studies in Hypersensitiveness. XIII. A Study of the Atopic Reagins, J. Immunol. **10**: 445, 1925.
7. Landsteiner, K., and Chase, M. W.: Experiments on Transfer of Cutaneous Sensitivity to Simple Compounds, Proc. Soc. Exper. Biol. & Med. **49**: 688, 1942.
Chase, M. W.: The Cellular Transfer of Cutaneous Hypersensitivity to Tuberculin, Proc. Soc. Exper. Biol. & Med. **59**: 134, 1945.

CHANGES IN WHITE BLOOD CELL AND BONE MARROW MORPHOLOGY AND SERUM PROTEIN FRACTIONS IN INDUCED AND SPONTANEOUS HYPERSENSITIVITY STATES

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FOR many years, investigators have been aware of the important role played by various elements of the reticuloendothelial system in the immune response.[1-3] A delicate mechanism has been postulated for the maintenance of normal gamma globulins and for the production of so-called "immune" gamma globulins on demand. From detailed animal experimentation, it has become increasingly apparent that the prime cellular response to antigenic stimulation lies somewhere within the lymphocyte-plasma cell system,[4, 5] but it is impossible at this stage of our knowledge to define precisely the role of either type of cell with regard to immunity, sensitivity, or protein production. Fagraeus[6] and others[7, 8] seem to have demonstrated, in tissue culture, that active antibody production is a function of the developing plasma cell. On the other hand, Daugherty and Harris have produced much convincing evidence that the lymphocyte is primarily involved in the response to antigenic stimuli.[2] These experiments, however, were performed on animals; the results, therefore, must be applied to human beings with reservations.

We first observed the changes to be discussed in the peripheral blood of a patient suffering from actue serum sickness with fever, urticaria, arthralgia, joint swelling, lymphadenopathy, and splenomegaly, following the subcutaneous administration of 1,500 units of antitetanus serum prophylactically. In a differential count, 30 per cent of the white blood cells were adult plasma cells, and there was an increase in the proportion of atypical young lymphocytes in a total count of 12,000 cells per cubic millimeter. In one week, after steroid therapy, all these findings had disappeared. Such a cellular response has been reported in the blood and bone marrow in states as diverse as experimental immunization and hyperimmunization of animals,[5, 6] human hypersensitivity to para-aminosalicylic acid and sulfonamides,[9-11] infection with rubella,[21] and acute stages of rheumatic fever.[13] Although changes in total white blood cell count and cell morphology were noted by von Pirquet and

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Schick[14] in the original monograph on serum sickness, neither they nor more recent writers made more than passing mention of such changes; nor did they give any indication of their incidence or severity.[15-17]

MATERIALS AND METHODS

This investigation was carried out on sixty-six patients who developed hypersensitivity to 1,500 units of prophylactic antitetanus serum. None received therapeutic doses. All reactions occurred between the sixth and tenth days after injection, and varied from mild urticaria to full-blown serum sickness requiring hospitalization. In seven of the latter, the studies were repeated after adrenal steroid therapy. All these patients had fever, generalized urticaria, arthralgia, and joint swelling, and five had generalized lymphadenopathy and slight splenomegly. Three control groups were included: (1) ten patients who had received antitetanus serum seven days previously without developing symptoms; (2) twenty-four spontaneously allergic persons, with and without symptoms; and (3) ten normal nonallergic persons.

Skin tests to antitetanus serum were done prior to its prophylactic administration on all reactive patients and on the ten nonreactive controls, and were repeated in one week in the latter group.

Blood studies included a total white blood count and a differential count made on 200 cells of capillary blood stained with Wright's stain; the counts were made by two different observers using the same slide. Peripheral smears were also stained with methyl green-pyronine and examined for the presence of pyroninophilia.

Serum proteins were measured by fractionation with sodium sulfate in thirty-one patients and by electrophoretic separation in thirty-five patients. The latter was performed in a Spinco Model R paper electrophoresis system, using Veronal buffer at pH 8.6 and ionic strength 0.1, in a Durrum cell. The patterns were stained with bromphenol blue, by the technique described in the Spinco manual, and protein densities were recorded on squared paper, by passage through the Spinco Analytrol. The different protein components were then expressed in relative percentages of the total protein present, as calculated from the specially calibrated paper provided for use with this apparatus. Each serum was examined in duplicate, and the average values were used for purposes of comparison.

Bone marrow aspiration was performed with a Turkel needle on six patients with severe antitetanus serum reactions. The smears obtained were stained with Wright's stain for routine counting and with methyl green-pyronine.

RESULTS

Peripheral Blood.—

Group A:

1. *Induced hypersensitivity with symptoms.* Fifty-six of the sixty-six patients had a white blood count over 10,000 (average 11,600) with an increased proportion of polymorphonuclear leukocytes, toxic granulation, and a slight

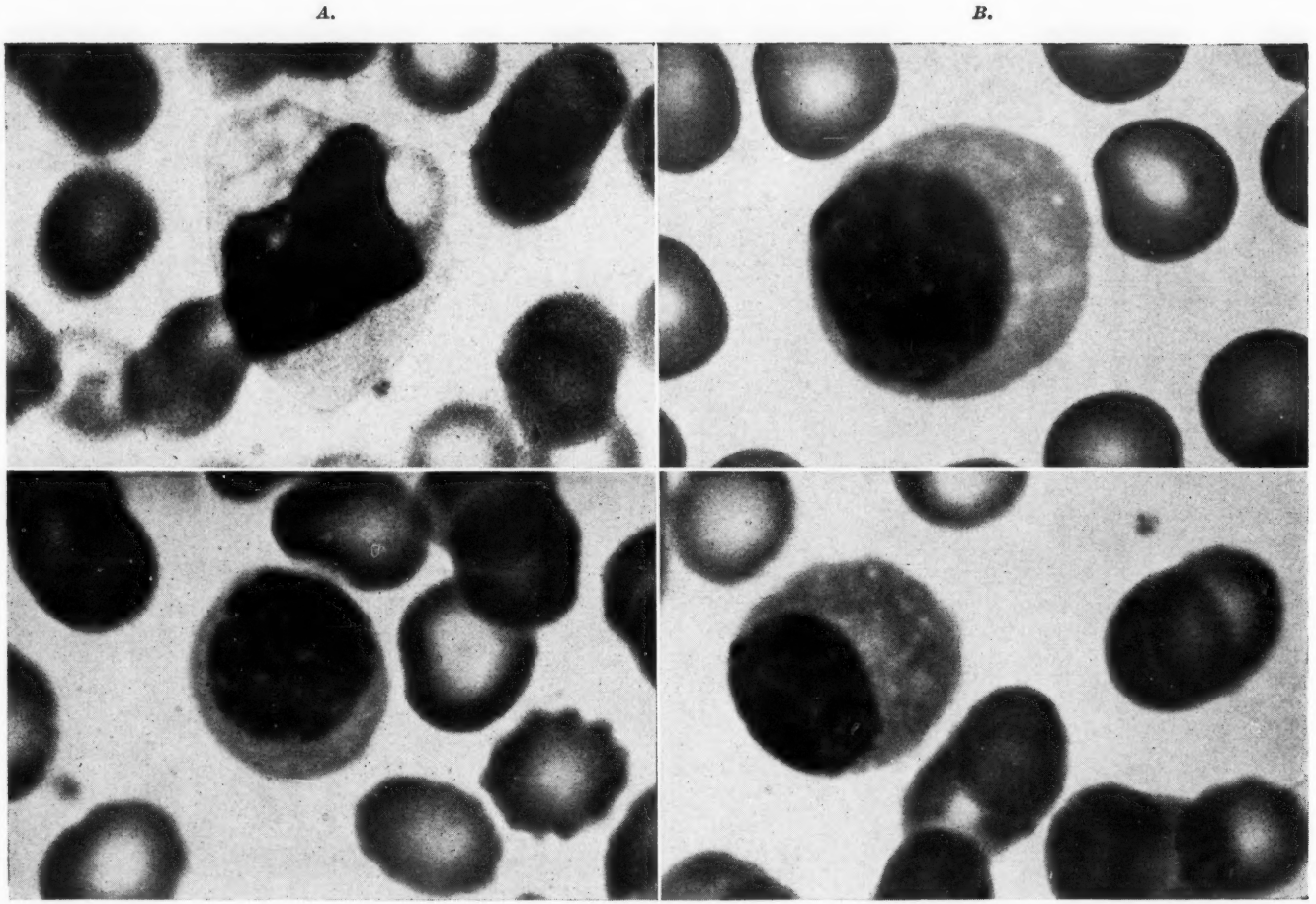

Fig. 1.—Morphologic changes in lymphocytes in the peripheral blood of patients with induced hypersensitivity. *A*, Atypical or "virus" type of lymphocyte; *B* and *C*, transitional forms of lymphocytes; *D*, adult plasma cell. (Oil-immersion magnification.)

shift to the left in the myeloid series. Only two patients had an eosinophilia of over 5 per cent. There was no consistent change in the proportion of mononuclear cells, but sixty-four patients did show definite morphologic alterations in that series. The most commonly seen abnormal cell was a large lymphocyte with abundant pale blue cytoplasm, frequently vacuolated, and a large slightly irregular nucleus (Fig. 1, *A*). These corresponded to the so-called "atypical lymphocyte" (or Downey cell) seen in many virus infections. There also were many smaller lymphocytes with less abundant, deeply basophilic cytoplasm, and the regular darkly staining nucleus of an adult lymphocyte.

Proceeding from these cells, and merging indistinguishably into adult plasma cells, it was possible to demonstrate a progressive series of changes in the nucleus and cytoplasm. Any subdivision of this intermediate group of cells would be arbitrary and artificial. They were larger than adult lymphocytes with intensely basophilic cytoplasm and large, dark nuclei with reticular chromatin, which was sometimes irregularly clumped (Fig. 1, *B* and *C*). As high as 8 per cent plasma cells were found, although about 2 per cent was the usual number (Fig. 1, *D*). Occasional proplasmacytes could be identified, but no plasmablasts were seen. There were no changes in the red blood count or platelets. Adrenal steroid therapy failed to alter cell morphology in any of the seven patients studied before and after medication. No significant changes occurred in differential counts, although occasional "smudge" cells were seen in all cases.

2. *"Induced hypersensitivity" without symptoms.* The ten patients who received antitetanus serum without reaction exhibited similar morphologic alteration in the lymphocyte series, but to a lesser degree and without any quantitative change in total white blood count or differential count. Their white counts and smears had been normal prior to the administration of serum.

Group B:

1. *Spontaneous hypersensitivity with symptoms.* Six patients with severe intrinsic (or infective) asthma and six with angioedema, urticaria, or hay fever exhibited morphologic changes in their lymphocytes and alterations in the differential counts which were similar to, but less marked than, those observed in patients with induced hypersensitivity, whether on steroid therapy or not. No adult plasma cells were seen. These changes were unrelated to the degree of eosinophilia present.

2. *Spontaneous hypersensitivity without symptoms.* Twelve asymptomaic patients with clinically controlled allergic rhinitis showed no such cellular alterations, although they all had a mild (3 to 4 per cent) eosinophilia. All were on hyposensitization therapy.

Bone Marrow.—The bone marrow was examined in six cases of severe generalized serum sickness. Although all the marrows examined showed a slight increase in the number of lymphocytes present with the same morphologic changes as those seen in the peripheral blood, none contained an increased

Fig. 1.—Morphologic changes in lymphocytes in the peripheral blood of patients with induced hypersensitivity. *A*, Atypical or "virus" type of lymphocyte; *B* and *C*, transitional forms of lymphocytes; *D*, adult plasma cell. (Oil-immersion magnification.)

number of plasma cells above the accepted normal peak of 6 per cent. Except for slight myeloid stimulation, there were no other changes in the bone marrow.

Serum Proteins.—The serum proteins of thirty-one patients with reactions to antitetanus serum were studied solely by salting out. Although there was a relatively constant elevation of euglobulin and 13.5 per cent globulin with depression of pseudoglobulin II, most marked in those patients with definite cellular changes, this relationship was by no means consistent. In an effort to find some closer relationship between alterations in cell morphology and serum protein pattern, paper electrophoresis was used to separate the proteins of the last thirty-five patients of the series and the control groups.

Serum albumin:

1. *"Induced hypersensitivity."* As can be seen from Fig. 2, patients in this group, with or without symptoms, had low relative serum albumin values. This shift was accompanied by trailing of the frequency curve to the left below the normal range, and was less marked in those patients who did not develop symptoms.

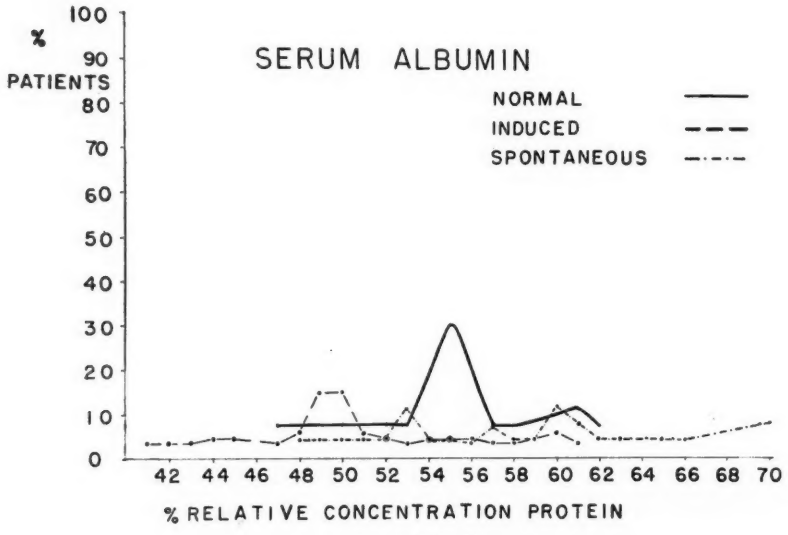

Fig. 2.

2. *Spontaneous hypersensitivity.* Patients with spontaneous hypersensitivity, with and without symptoms, showed no such decrease, but values were scattered and distributed mainly to the right of the frequency curve above the normal range.

Serum gamma globulin:

1. *"Induced hypersensitivity."* As can be seen from Fig. 3, patients in this group showed elevated gamma globulin values, with a shift to the right of normal values, and a trailing off into higher levels. In addition, occasional high values were obtained for beta and alpha$_2$ globulins. An abnormal globulin peak was demonstrated in only two patients, and in both it lay between

beta and alpha$_2$, and was present in considerable amount. The increase in globulin was slight in those patients who received antitetanus serum but did not develop signs or symptoms of hypersensitivity. In four of the ten patients the globulin change was not statistically significant.

Effects of Steroid Therapy.—Seven patients with severe serum sickness were admitted to the hospital and treated with intravenous hydrocortisone hemisuccinate, followed by oral prednisone. The dosage was adjusted to control the patients' symptoms and was tapered off gradually. Once the clinical evidence of hypersensitivity had subsided, the cell counts, smears, and protein fractions were re-examined. No significant change in either cells or serum proteins was found in any of these patients.

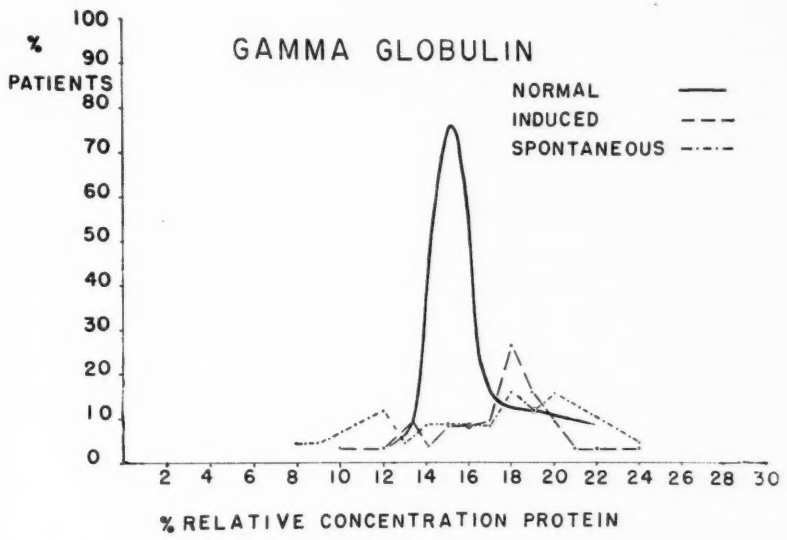

Fig. 3.

Skin Testing.—Only eight of the subjects who subsequently exhibited a reaction to antitetanus serum had a positive skin test prior to its administration. None were retested. Five control patients were skin tested before and one week after being given 1,500 units of serum, and none of these tests became positive.

DISCUSSION AND CONCLUSIONS

The results obtained in this short-term clinical experiment have provided evidence that the hypersensitivity response in man, as in animals, is mediated through the lymphocyte-plasma cell system. The most consistent cellular response in both induced and spontaneous hypersensitivity was an alteration of lymphocyte morphology to resemble the plasma cell. It is significant that adult plasma cells appeared in only the patients with induced serum sickness; this suggests that such reactions may represent quantitative differences in tissue response to challenge, rather than any qualitative alteration in the mechanism itself.

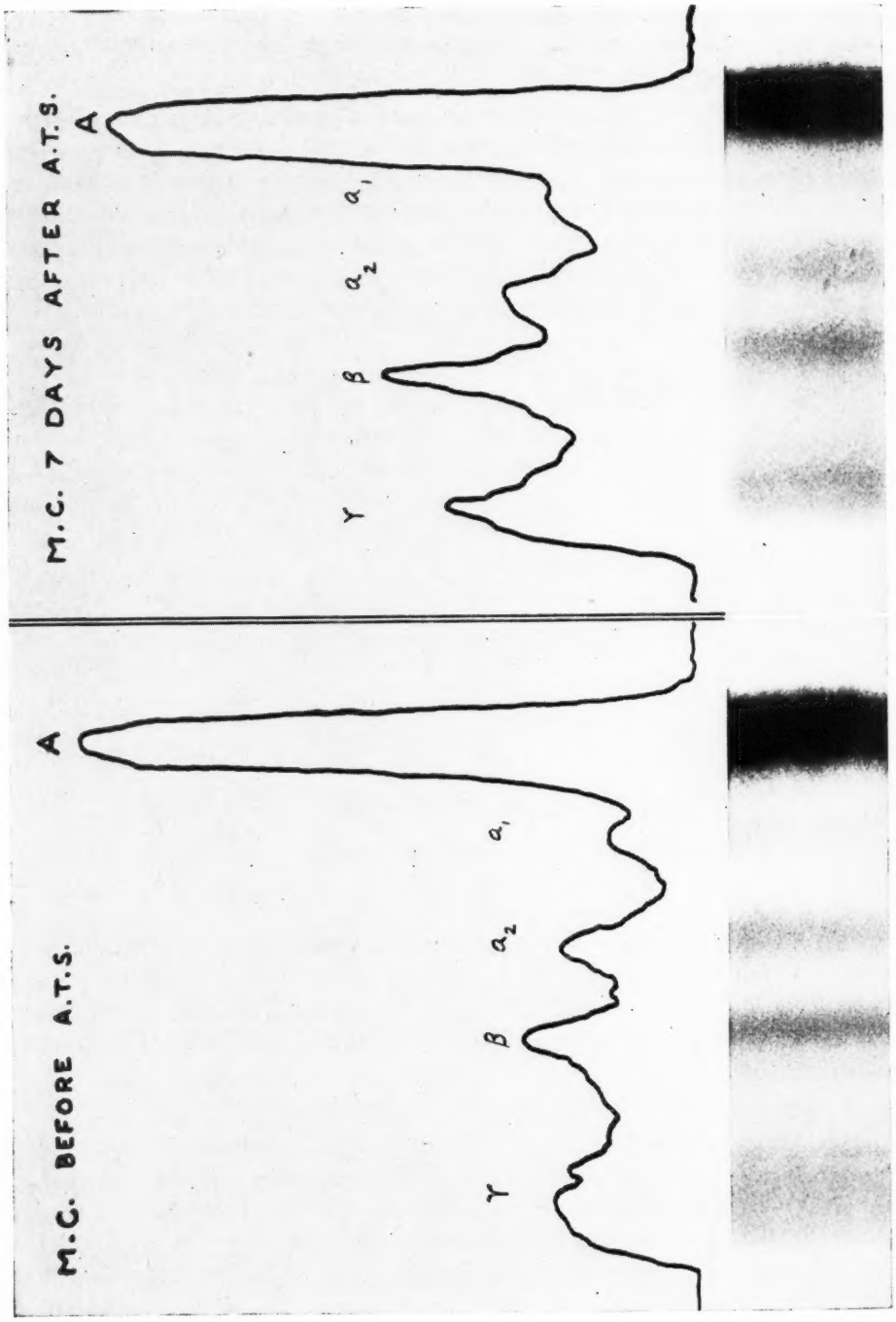

Fig. 4.—Electrophoretic patterns.

The bone marrows of six patients mirrored the changes in the peripheral blood, which conforms with the present belief that the plasma cell and lymphocyte are formed primarily in lymph nodes and other elements of the reticuloendothelial system, and not in the marrow.

No attempt was made to divide the cells into two groups on a morphologic basis. The trend certainly was toward an increased population of a plasma cell type. This was supported by a slight but definite increase in pyroninophilia of the more basophilic of these cells without evidence of immaturity, such as nuclear changes. We could conclude only that both cell types were involved in an interrelated reaction to an antigenic stimulus.

The cell changes in the induced hypersensitivity states were accompanied by an increase in circulating gamma globulins, and very occasionally by increased beta and alpha globulins (particularly $alpha_2$). Although there was no precise correlation between the clinical severity of the reaction and the changes in cells or proteins, the most marked alterations in both were seen in symptomatic as opposed to "asymptomatic" hypersensitivity.

The significance of the opposing changes in serum albumin in the two groups of patients is unknown. Long[18] has emphasized that serum protein values at any given time are representative of biologic responses to many factors, including infection, nutrition, and endocrine functions. Although overt infection was avoided when choosing subjects to be included in the spontaneously hypersensitive group, many had had symptoms for years, and the factors determining serum albumin levels may have been multiple, apart from hypersensitivity per se.

The gamma globulin levels were increased in both types of hypersensitivity. This was most definite, and constant, in the induced type in which there was a well-defined frequency peak to the right of the normal curve. Occasional isolated peaks occurred in beta and $alpha_2$ components (Fig. 4). A less well-defined shift to the right could be demonstrated in the spontaneous type of sensitivity, but no other globulin peaks were present. Values for gamma globulin in both groups were widely distributed, but the spread was more marked in the spontaneous group.

Fig. 4.—Electrophoretic patterns.

Interpretation of these results without measurement of biologic antibody activity in the various globulin fractions is, unfortunately, mere speculation. However, it seems reasonable to consider the scattered globulin peaks and the shift of globulin values to the right of the normal range as reflections of antibody activity whether due to reagin or to other types of antibodies. The failure to produce a positive skin test in the control patients is reflected in the minor cellular and globulin changes in these patients and is possibly indicative of minimal antibody response on their part.

These findings suggest that the changes produced in blood cells after stimulation with antitetanus serum are similar to those which occur in patients with a tendency to develop spontaneous allergic reactions. The hyperimmune response with clinical serum sickness differs only in the degree of

cellular reaction. The widespread globulin changes found in these patients might represent an excessive response of the body's immune mechanism, with production of a variety of qualitatively different antibodies.

SUMMARY

1. Changes in the lymphocyte-plasma cell series in the peripheral blood and bone marrow of patients with induced and spontaneous hypersensitivity have been described.

2. Adrenal steroid therapy, sufficient to suppress symptoms, did not affect the changes observed.

3. Variations in serum protein fractions in both types of hypersensitive states have been noted. These primarily involved the gamma globulins in the induced group, but alterations in all globulin components were noted in the spontaneous group.

The authors would like to express their appreciation for the advice and technical assistance given to them by Miss M. Grady, Miss R. E. Porrett, and Mr. J. S. Wilson of the Haematology and Biochemistry Departments of the Toronto Western Hospital.

REFERENCES

1. Barr, David: The Function of the Plasma Cell, Am. J. Med. **9**: 277, 1950.
2. Chase, Merrill W., Dameshek, William, Haberman, Sol, Samter, Max, and Squier, Theodore: The Role of the Formed Elements of the Blood in Allergy and Hypersensitivity, J. ALLERGY **26**: 219, 1955.
3. Fadem, Robert S., and McBirnie, John E.: Plasmacytosis in Disease Other Than the Primary Plasmacytic Diseases, Blood **5**: 191, 1950.
4. Keuning, F. J., and van der Slikke, L. B.: The Role of the Immature Plasma Cells, Lymphoblasts, and Lymphocytes in the Formation of Antibodies, as Established in Tissue Culture Experiments, J. Lab. & Clin. Med. **36**: 167, 1950.
5. Kolouch, Fred: The Origin of Bone Marrow Plasma Cells Associated With Allergic and Immune States in the Rabbit, Proc. Soc. Exper. Biol. & Med. **39**: 147, 1938.
6. Fagraeus, Astrid: The Plasma Cellular Reaction and Its Relation to the Formation of Antibodies In Vitro, J. Immunol. **58**: 1, 1948.
7. Moeschlin, S., Pelaez, P. R., and Hugentobler, F.: Experimental Investigations of the Relationship Between Plasma Cells and Antibody Formation (Phase Contrast Microscope), Acta Haemat. **6**: 321, 1951.
8. Mota, Ivan, Yoneda, Sakae, and Rabinovitch, Iria: Plasmal Reaction in the Blood and Haemopoietic Organs, Proc. Soc. Exper. Biol. & Med. **81**: 338, 1952.
9. Cannemeyer, W., Thompson, J. R., and Lichtenstein, M. R.: Severe PAS Hypersensitivity: Blood and Bone Marrow Studies, Blood **10**: 62, 1955.
10. Mellette, S. J., and Agress, H.: Toxic Reaction to PAS and Izoniazid Accompanied by Leukopenia and Atypical Lymphocytosis, Am. Rev. Tuberc. **69**: 824, 1954.
11. Steininger, W. J., Klopfenstein, M. D., and Woodruff, S. S.: Fatal Allergic Reaction to PAS, Am. Rev. Tuberc. **69**: 451, 1954.
12. Whitby, L. E. H., and Britton, C. J. C.: Disorders of the Blood, ed. 7, London, 1953, J. & A. Churchill, Ltd., p. 103.
13. Good, Robert A., and Campbell, Berry: Relationship of Bone Marrow Plasmacytosis to the Changes in Serum Gamma Globulin in Rheumatic Fever, Am. J. Med. **9**: 330, 1950.
14. Von Pirquet, C. F., and Schick, B.: Serum Sickness, Baltimore, 1951, Williams & Wilkins Company.
15. Pariser, Sanford, Sucker, Richard, and Meyer, Leo: Haematologic Changes Associated With the Immune Response in Man, Acta med. scandinav. **144**: 201, 1952.
16. Randolph, Theron, and Gibson, Elizabeth: Blood Studies in Allergy. II. The Presence in Allergic Disease of Atypical Lymphocytes and Symptoms Suggesting the Recovery Phase of Infectious Mononucleosis, Am. J. M. Sc. **207**: 638, 1944.
17. Kallos, Paul (Editor): Progress in Allergy, Boston, 1955, Little, Brown & Company, Vol. IV, p. 31 (M. G. Bohrod).
18. Long, D. A.: The Pathogenesis of Rheumatic Fever, The Lancet **CCLXVI**: 529, 1954.

LOCALIZATION OF BLOCKING ANTIBODY IN SERA OF RAGWEED-SENSITIVE INDIVIDUALS BY STARCH ELECTROPHORESIS

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IN A recent report from this laboratory[1] it was shown that sera of ragweed-sensitive persons could be separated into electrophoretically distinct serum fractions by starch electrophoresis, and that the skin-sensitizing activity was associated with the gamma$_1$ and/or gamma$_2$ globulins. All other serum fractions were found to be devoid of reaginic activity.

In continuation of our studies on antibody-like factors in allergic sera, starch electrophoresis was used in the present investigation for the localization of blocking antibodies in the sera of treated allergic persons. Since the initiation of the present work, two independent reports[2, 3] have been published recently on the localization of the blocking antibody to ragweed pollen. Cooke and associates,[2] using the combined methods of ammonium sulfate fractionation, electrophoresis-convection, and free electrophoresis with the aid of the preparative macro-Tiselius cell, and Loveless and Cann,[3] using electrophoresis-convection, were able to demonstrate that the bulk of the blocking antibody was associated with gamma globulins. However, the possibility of some blocking activity being associated with other serum proteins could not be unequivocally excluded. The results of our study, presented in this article, indicate that blocking antibody is associated exclusively with gamma globulins.

EXPERIMENTAL

Blocking sera were obtained from six ragweed-sensitive patients who had been given an intensive course of injections of whole ragweed pollen extract for a period of two to three months prior to bleeding. About 100 ml. of blood was collected from each patient. The blood was processed in the usual way.[1]

Zone Electrophoresis.—Since the present study constitutes only a preliminary survey as regards the localization of the blocking antibody, the sera were separated into pooled "peak fractions,"[1] namely, into the clearly delineated albumin, and α_1-, α_2-, β-, γ_1-, and γ_2-globulin fractions, and not into the different serum subfractions (40 to 60 eluates) as was done in the study of the

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skin-sensitizing antibody. The procedure used was otherwise identical in all respects to that described previously.[1] The pooled eluates, constituting the different, electrophoretically distinct, serum fractions were individually Seitz filtered into sterile Visking tubings and concentrated by pervaporation to final volumes of about 6 to 8 ml. Pervaporation was alternated with dialysis against 0.9 per cent saline solution in order to avoid excessive concentration of salts in the Visking tubings. For the same reason, in some experiments the sterile contents of the Visking tubing were dialyzed against distilled water or tap water prior to pervaporation. In the latter cases pervaporation was performed without interruption until the desired final concentration was reached. The concentrated serum fractions to be skin tested were then dialyzed against saline for about twenty-four hours and transferred into sterile vials through Swinny-Seitz filters. The sterile fractions and whole sera were stored at 4° C.

Tiselius Electrophoresis.—This was used for the analysis of the whole sera. The method as applied in this laboratory was fully described previously.[1] The individual pooled "peak fractions" were not recharacterized by Tiselius electrophoresis, since it was shown earlier[1] that the designation of the various serum components on the basis of the protein distribution curve obtained by starch electrophoresis corresponded closely to that commonly accepted for free electrophoresis.

Detection and Titration of Blocking Antibody.—The method used in this study was essentially similar to that described by Loveless[3, 4] and by Cooke and associates.[2] It depends primarily on the ability of blocking antibody to combine or inactivate in vitro a known amount of the corresponding allergen. In this method a known amount of reagin is used as internal indicator. The method consisted of two stages: (1) titration of the reagin content in an aliquot of serum containing only skin-sensitizing antibody with a known amount of allergen and (2) titration of the blocking antibody. To avoid complications due to the presence of reagin, which is normally present in addition to blocking antibody in sera of treated allergic persons, the former was inactivated prior to the titration of blocking antibody in the whole sera and in the serum fractions by heating at 56° C. for periods of as long as six hours.

Stage 1: A reaginic serum, obtained from an untreated ragweed-allergic patient, was diluted with saline so that 1 ml. of the solution contained sufficient reagin to neutralize approximately 200 units of ragweed pollen.* This solution will be referred to hereafter as R. Six sites on the back of a normal nonsensitive person were injected with 0.05 ml. of solution R. Twenty-four hours later these sites were injected with 0.05 ml. of ragweed pollen extract of varying concentrations (for example, from 50 to 300 units per milliliter). Within twenty to forty minutes, typical wheals and flares developed at each site, the minimum reaction being given by the sites injected with the lowest

*One unit corresponds to 10^{-5} mg. nitrogen.

dose of ragweed pollen. However, no direct relationship was observed between the amount of ragweed injected and the extent of reaction.* On the third day all sites were injected with 0.05 ml. of a concentrated ragweed pollen extract (500 to 600 units per milliliter). The sites which had received previously (on the second day) amounts of ragweed in excess of that required for neutralizing the reagin content in 0.05 ml. of solution R did not give any reactions. The sites which had received smaller amounts of the allergen than that required for neutralization gave positive reactions. The true neutralizing dose was considered to correspond to the average between the lowest dose of ragweed injected on the second day, which was just short of neutralizing all the reagin, and the next higher dose which had reacted with all the reagin. The scheme of a typical experiment for the titration of reagin in solution R is given in Table I. Additional control sites were injected on the first day with saline and on the third day with the ragweed solution containing 500 units per milliliter. The sites were marked with carbolfuchsin to facilitate their recognition. All tests were done in triplicate on the same subject. Each experiment was repeated on several subjects.

TABLE I

		SITES					
		1	2	3	4	5	6
First day	Sensitization with R*	0.05 ml.	0.05 ml.	0.05 ml.	0.05 ml.	0.05 ml.	0.05 ml.
Second day	Injection of allergen†	50 u./ml.	100 u./ml.	150 u./ml.	200 u./ml.	250 u./ml.	300 u./ml.
Third day	Injection of allergen†	500 u./ml.	500 u./ml.	500 u./ml.	500 u./ml.	500 u./ml.	500 u./ml.

*R represents reaginic serum diluted 1:100.
†0.05 ml. used for each injection, 1 u. = 10^{-5} mg. nitrogen.

Stage 2: For the detection of blocking antibody, the following procedure was used: An aliquot of the whole serum or serum fraction suspected of containing blocking antibody was mixed in a sterile vial with a calculated amount of allergen (An) and incubated at 4° C. for twenty-four hours (mixture An-B).† An appropriate volume of the reaginic serum‡ used in Stage 1, corresponding to that which would be neutralized by An units of the allergen, was then added to the mixture "An-B" and the vial was allowed to stand at room temperature for about one hour. Subsequently, 0.05 ml. of the combined mixture was injected into the skin of a nonallergic person. Control sites were injected with a mixture containing the same amounts of reagin and ragweed as in the test mixture, the only difference being that the blocking serum was replaced by saline. Some of the sites gave immediate reactions which were ignored since they could not be graded. Twenty-four hours later both test and control sites were injected with excess allergen. The control sites gave no reactions, since there was no free reagin left to react with the additional

*This problem is fully discussed elsewhere.[5]
†The solutions were filtered through a Swinny-Seitz filter.
‡The same reaginic serum was used in all blocking experiments.

allergen injected on the second day. On the other hand, sites containing blocking antibody gave a wheal and erythema, thus demonstrating that some of the allergen An had been "blocked" during incubation and that reagin was still available for further reaction with the allergen.

For the titration of blocking antibody, a series of mixtures containing the blocking serum in halving dilutions and constant amounts of reagin and ragweed (the latter two in neutralizing amounts with respect to each other) were made up as described above. Then 0.05 ml. of these mixtures was injected into the skin of a test subject. After twenty-four hours all sites were injected with excess allergen. The sites sensitized with mixtures containing the highest amount of blocking antibody gave the strongest reaction. The other sites, containing blocking antibody in decreasing amounts, gave smaller and graded reactions. The blocking titer was given by the $(\frac{1}{2})^n$-th dilution of the blocking serum (where n represents the number of half-dilutions) which still gave a detectable reaction. Thus, using the same amount of reagin and the corresponding neutralizing dose of allergen, the degree of blocking ability could be established for a series of blocking sera. All tests were done in triplicate and on more than one subject. A typical experiment for the titration of blocking antibody is illustrated in Table II.

TABLE II. PROCEDURE FOR TITRATION OF BLOCKING ANTIBODY

	TEST MIXTURES							
	1	2	3	4	5	6	7	8
2 ml. of blocking serum in dilutions	1:1	1:2	1:4	1:8	1:16	1:32	1:64	0 ml.
Volume of saline	0	0	0	0	0	0	0	2 ml.
Allergen solution*	1 ml.	1 ml.	1 ml.	1 ml.	1 ml.	1 ml.	1 ml.	1 ml.
All mixtures incubated at 4° C. for 24 hours, followed by addition of reagin solution.								
Reagin solution*	1 ml.	1 ml.	1 ml.	1 ml.	1 ml.	1 ml.	1 ml.	1 ml.
After incubation for 1 hour at room temperature, 0.05 ml. of each mixture was used for Prausnitz-Küstner test.								

*The allergen and reagin solutions were used in neutralizing concentrations with respect to each other, as determined in titration experiments. Since both solutions were diluted four-fold in the preparation of these test mixtures, their concentrations were four times as high as those used in titration experiments.

The allergen solutions used for making up the mixture "An-B" and for challenging the sensitized sites consisted of a water-soluble ragweed pollen extract (WSR) in preliminary experiments. Since it caused some irritation and did not give consistently reproducible results, it was replaced by a ragweed pollen fraction, named AA_1+Pt* (prepared by zone electrophoresis using filter paper as a supporting medium) which gave well-defined reactions without irritation. The justification for using AA_1+Pt instead of WSR was afforded by the fact that the same blocking titer was obtained with either allergenic solution. The sera separated by starch electrophoresis were heated just prior to the titration of the blocking antibody. The concentrated fractions

*We are indebted to Mr. M. Richter of this laboratory for the AA_1+Pt ragweed pollen fraction. The preparation of this fraction is described elsewhere.[6]

were tested for reaginic content before determination of their blocking titer. The fractions containing reagin were heated at 56° C. for a period not longer than necessary to inactivate reagin.

RESULTS AND DISCUSSION

The results of free electrophoresis for the six sera studied are given in Table III, together with our values for normal sera. The extent of reaginic activity of the six sera, as measured by the degree of dilution which still gave a positive skin test, is represented in Table IV. It is interesting to note that

TABLE III

EXPERIMENT NO.	SERUM	T.P.* GM./100 ML.	ALB. PER CENT	ALB. GM./100 ML.	α_1 PER CENT	α_1 GM./100 ML.	α_2 PER CENT	α_2 GM./100 ML.	β PER CENT	β GM./100 ML.	γ PER CENT	γ GM./100 ML.
E 1064	A	7.20	63.17	4.55	3.49	0.25	7.56	0.54	13.27	0.95	12.50	0.90
E 996	Sh	7.32	61.89	4.53	4.93	0.36	5.54	0.40	14.09	1.03	13.55	0.99
E 947	P	6.84	52.70	3.60	3.38	0.23	7.97	0.54	13.58	0.93	22.36	1.53
E 1266	W I	7.98	61.22	4.88	4.61	0.37	8.37	0.67	12.06	0.96	13.73	1.09
E 1414	W II	7.32	62.97	4.61	4.62	0.34	6.65	0.47	14.77	1.08	10.99	0.80
E 1412	D II	7.26	51.86	3.76	5.50	0.40	11.78	0.85	15.58	1.13	15.27	1.11

NORMAL SERA

	T.P. GM./100 ML.	ALB. PER CENT	ALB. GM./100 ML.	α_1 PER CENT	α_1 GM./100 ML.	α_2 PER CENT	α_2 GM./100 ML.	β PER CENT	β GM./100 ML.	γ PER CENT	γ GM./100 ML.
22 Males											
Average	7.93	59.46	4.73	4.68	0.36	8.60	0.69	12.92	1.06	14.33	1.08
S.D.	±0.56	±3.16	±0.31	±1.81	±0.06	±1.43	±0.16	±1.27	±0.12	±22.46	±0.19
14 Females											
Average	7.92	58.40	4.69	5.17	0.42	9.81	0.72	12.42	1.00	14.18	1.08
S.D.	±0.71	±2.31	±0.39	±0.73	±0.07	±0.98	±0.13	±1.18	±0.11	±1.76	±0.17

*T.P. refers to total protein, determined refractometrically.

the first, serum A, did not give a positive Prausnitz-Küstner reaction, although a strongly positive skin test was given by the patient who also had clinical symptoms characteristic of ragweed hay fever. The other five sera gave strongly positive reactions in normal skin.

TABLE IV

SERUM	REAGIN TITER	BLOCKING TITER
A	0	1:4
Sh	1:1,000	1:4
P	1:10,000	1:8
W I	1:1,000	1:32
W II	1:1,000	1:16
D II	1:1,000	1:64

All six sera were shown to contain blocking activity after treatment. Their blocking titers are given in Table IV. The first two sera, A and Sh, exhibited only weak blocking ability, which could not be detected in dilutions higher than 1:4. The other four sera had reasonably good blocking titers.

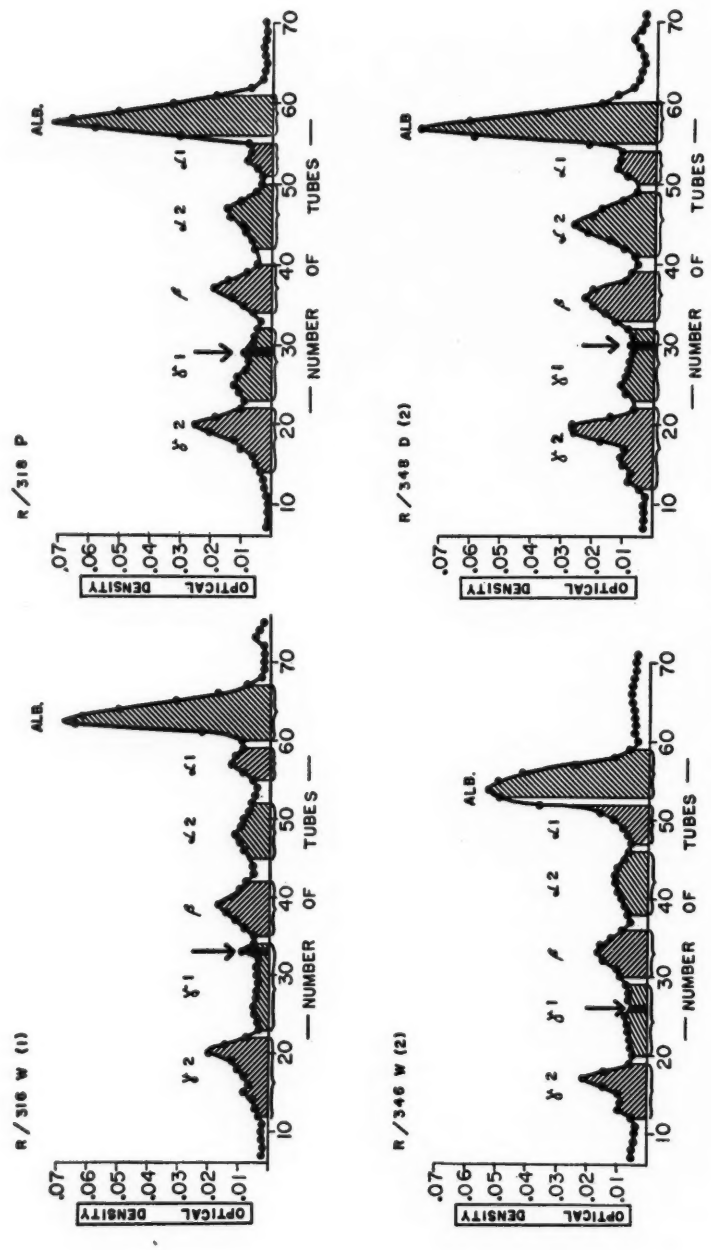

Fig. 1.—Separation of blocking sera.

The electrophoretic separation of four sera by starch electrophoresis is illustrated in Fig. 1. Since the primary aim in the present study was to develop a method for the demonstration of blocking antibody in the serum fraction(s) with which it is associated, the starch blocks were divided into only five or six large fractions without eliminating the possible contamination due to serum proteins present in the interzonal regions. Blocking activity could not be clearly demonstrated in any of the fractions of the two weakly blocking sera, A and Sh. In all the other four sera, however, blocking activity was found to be associated with the gamma$_2$ globulins. In addition, in one of the four sera, namely, D II, some blocking activity was also detected in the gamma$_1$ globulins. All other serum fractions were found to be completely devoid of any blocking activity. Our results confirm the previous findings of Cooke and associates[2] and Loveless and Cann[3] and demonstrate that blocking activity in sera of ragweed-sensitive patients is confined to γ-globulins only, mainly to the slow γ_2-globulins.

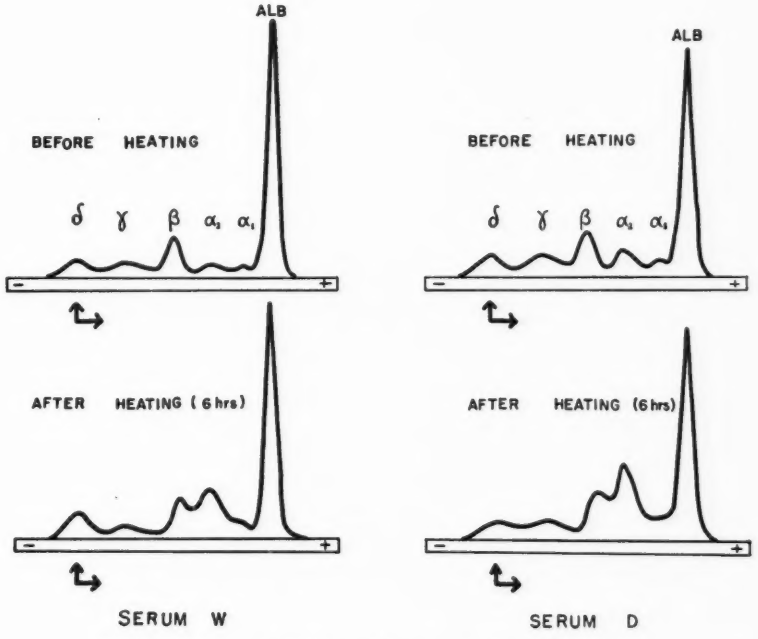

Fig. 2.—Effect of heating on human serum (56 degrees).

In sera P, Sh, and W I, reaginic activity was found to be associated only with the γ_1-globulins. These findings are similar, to some extent, to those of Kuhns,[7] who has shown that skin-sensitizing antibodies and blocking nonprecipitating antibodies to diphtheria toxin are found in the γ_1- and γ_2-globulins, respectively.

In this connection, it is also worth mentioning that Cooke and associates,[2] using ammonium sulfate fractionation, showed that the skin-sensitizing and blocking antibodies to ragweed were associated with two different fractions, isolated by precipitation at 55 per cent and 33.3 per cent ammonium sulfate,

respectively. In our own experience, serum proteins precipitated at 33.3 per cent ammonium sulfate contain mainly γ_2-globulins, while the additional fraction precipitated at 55 per cent ammonium sulfate contains the faster moving α-, β-, and γ_1-globulins in addition to small amounts of γ_2-globulins.

The reagin present in the sera to be fractionated was inactivated by heating only after separation on the starch blocks, since, as one would expect,[8] heating at 56° C. for periods as long as six hours caused considerable alteration of some serum proteins, as demonstrated by electrophoretic analysis. This is illustrated in Fig. 2. As can be seen, the β-globulins of the whole sera were affected, on heating, to a larger extent than any of the other serum proteins, the net effect manifesting itself by an increase in mobility of the altered proteins. The sera remained clear after heating for periods of as long as six hours. Electrophoretic analysis of sera heated for varying shorter times showed that the alteration of the β-globulins was a gradual process. These findings confirm other results obtained in this laboratory,[9] suggesting that the β-globulins, in all probability the β-lipoproteins, are the most labile components of human serum, at least as determined by electrophoresis.

The complexity of the test for blocking antibody is self-evident. The main source of error in determining the blocking titer lies in establishing the correct dose of allergen necessary to neutralize the reagin used as the indicator of the titration. Incorporation in the mixture "An-B" of larger amounts of allergen than necessary will lead to an erroneously low blocking titer or to complete failure in detecting any blocking antibody. Conversely, addition of lower quantities of allergen will give an apparently higher blocking titer than the true one. However, it ought to be stressed that, in spite of these difficulties, the results obtained with any given series of test mixtures were independent of the normal skin used. Attempts at increasing the sensitivity of the test for blocking antibody are being made and will be reported elsewhere.

SUMMARY

Using starch electrophoresis, blocking activity of four sera of treated ragweed-sensitive patients was demonstrated to reside mainly in the slow-moving gamma globulins. No blocking antibodies were detected in other serum fractions. The method of detection and titration of blocking antibodies is described in detail.

We should like to acknowledge the skillful technical assistance of Mr. P. L. Rojowski, Mr. S. Kennedy, and Mrs. A. Elovic in these experiments.

REFERENCES

1. Sehon, A. H., Harter, J. G., and Rose, B.: The Localization of Skin-Sensitizing Antibody in the Sera of Ragweed-Sensitive Individuals by Electrophoresis, J. Exper. Med. **103**: 679, 1956.
2. Cooke, R. A., Menzel, A. E. O., Kessler, W. R., and Myers, P. A.: The Antibody Mechanisms of Ragweed Allergy, Electrophoretic and Chemical Studies. I. The Blocking Antibody, J. Exper. Med. **101**: 151, 1955.
3. Loveless, M. H., and Cann, J. R.: Distribution of "Blocking" Antibody in Human Serum Proteins Fractionated by Electrophoresis-Convection, J. Immunol. **74**: 329, 1955.

4. Loveless, M. H.: Immunologic Studies on Pollinosis. I. The Presence of Two Antibodies Related to the Same Pollen Antigen in the Serum of Treated Hay Fever Patients, J. Immunol. **38:** 25, 1940.
5. Richter, M., Harter, J. G., Sehon, A. H., and Rose, B.: Studies on Ragweed Pollen. III. Estimation of the Minimum Number of Allergens in the Water-Soluble Extract of Ragweed Pollen and a Critical Evaluation of the Neutralization Technique. (To be published in J. Immunol.)
6. Richter, M., Sehon, A. H., and Rose, B.: Studies on Ragweed Pollen. II. Fractionation of the Water-Soluble Extract of Ragweed Pollen by Zone Electrophoresis and the Characterization of the Fractions. (To be published in J. Immunol.)
7. Kuhns, W. J.: Immunologic Properties of a Form of Non-precipitating Diphtheria Antitoxin Which Does Not Sensitize Human Skin; Studies of Its Relation to the Inhibition Reaction, J. Immunol. **75:** 105, 1955.
8. Van der Sheer, J., Wyckoff, R. W. G., and Clarke, F. L.: An Electrophoretic Study of Heated Horse Serum, J. Immunol. **40:** 39, 1941.
9. Webb, T., Sehon, A. H., and Rose, B.: A Method of Isolation of Proteins From Dilute Solutions, Canad. J. Biochem. & Phys. **35:** 63, 1957.

STUDIES OF MATERIALS DERIVED FROM STAPHYLOCOCCUS

III. Protective Action of Gamma Globulin Against the Anaphylactoid Effects of a Staphylococcal Bacterial Fraction*

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SALINE extracts prepared in this laboratory from three strains of *Staphylococcus aureus,* washed free from culture filtrate, have been reported to cause anaphylactoid reactions in the isolated guinea pig ileum.[1, 2] This toxic effect was limited to a fraction precipitated at pH 3.5 (fraction A), which is probably a nucleoprotein.[1] In contrast to the anaphylactoid effect of bacterial extract and fraction A from pathogenic strains of bacteria, extract and fraction A from nonpathogenic staphylococci had no toxic properties.[1] Furthermore, the toxic activity of the extract was quite different from the toxic effect of culture filtrates of the same strains of *Staphylococcus aureus.*[2]

In the process of extending this work, the protective effect of horse antistaphylococcic serum upon the anaphylactoid reaction of toxic fraction A was studied. However, control studies revealed that *normal* horse serum had the same protective effect as the antiserum. The present article reports the results of investigations of this protective effect.

MATERIALS AND METHODS

One- to two-inch segments of fresh guinea pig ileum, suspended in a 5 ml. bath containing Tyrode's solution, maintained at 37° C. were used. The upper end of the segment was tied to a simple lever which recorded longitudinal movements of the segment on a smoked drum rotating at a speed of 2.5 cm. per minute.

In each experiment 0.2 ml. of serum, or equivalent doses of serum fractions, were added to the bath. Thirty seconds to one minute later, toxic fraction A was added and the record was continued for one and one-half minutes, to allow a contraction, if any, to occur. In order to assure complete removal of the serum or serum fractions, the bath was then rinsed three times, followed by five additions of 0.1 mcg. of histamine standard with three washes

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*Bibliography refrences 1 and 2 are considered Parts I and II, respectively, in this series.

between each addition. Toxic fraction A was then added, without serum or serum fractions, to demonstrate the anaphylactoid reaction described in previous reports.[1, 2]

In a few of the earlier experiments, rather than adding the materials separately, serum was first mixed with fraction A and incubated at 37 degrees for thirty minutes prior to addition to the bath. This prolonged incubation was found to be unnecessary.

A total of 110 segments of ileum from seventy-three guinea pigs were used (Table I). Sixty segments were used to study the protective effects of serum or gamma globulin against the toxic staphylococcal extract or fraction A, and the remaining fifty segments were used in control studies. These included investigation of the effect of substances other than whole serum or gamma globulin upon the toxic activity of bacterial extract or fraction A, as well as determination of the effect of serum or gamma globulin on the action of myotonic substances other than bacterial extract or fraction A.

TABLE I. DISTRIBUTION OF 110 EXPERIMENTS IN STUDY (73 ANIMALS)

SUBSTANCES STUDIED FOR PROTECTIVE EFFECT	MYOTONIC AGENTS						
	STAPHYLOCOCCUS AUREUS						
	WHOLE EXTRACT	FRACTION A	CULTURE FILTRATE	HISTAMINE	COBRA VENOM	ANAPHYLAXIS	TOTAL
Serum,* whole	24	7	1	8		1	41
Serum gamma globulin	6	12	5	3	2	8	36
Serum beta globulin	5						5
Serum alpha globulin	2	1					3
Serum albumin	3						3
Serum fibrinogen		1					1
Serum, whole, rat	6	5					11
Egg white	3						3
Egg yolk	2	2					4
Cobra venom	1						1
Glycerin	2						2
Totals	54	28	6	11	2	9	110

*Human, equine, bovine, rabbit, and guinea pig.

Bacterial extract, fraction A, and culture filtrate were prepared as described previously.[1-3] Culture filtrate was dialyzed against distilled water to remove histamine which is regularly present.

Naja nivea (cobra) venom was obtained in crystalline form* and was used in aqueous solution.

Human, horse, rabbit, guinea pig, and rat sera were used in various experiments.

*Kindly supplied by Dr. Charles N. Bogert, American Museum of Natural History. New York, N. Y.

The following serum fractions were used: bovine serum fibrinogen; albumin; alpha, beta, and gamma globulin,* pooled human gamma globulin,† and one individual human alpha and beta globulin sample.‡

In addition, 20 per cent aqueous solutions of egg white and egg yolk and a 25 per cent aqueous solution of glycerin were used for some of the control studies. Histamine standard contained 1.0 mcg. of histamine base per milliliter.

RESULTS

To demonstrate protection, two criteria must be fulfilled: (1) absence of contraction of the ileum following addition of bacterial extract or fraction A (F-10) to the bath containing serum, serum fractions, or controls, and (2) typical anaphylactoid response to bacterial extract or fraction A, after removal of serum, fractions, or controls, followed by tachyphylaxis (that is, loss of responsiveness to bacterial extract or fraction A after the initial contraction).

Experiments in which the reaction to extract or fraction A was unaffected by the presence of serum, fractions, or control materials were considered "no protection." Those in which only one of the two criteria was fulfilled were considered "undetermined." This classification was necessary because, unless a reaction to extract or fraction A could be demonstrated after removal of the protective substance, one could not be certain that the ileum segment was capable of response originally, a prerequisite for the demonstration of protection.

Whole serum or gamma globulin protected the isolated guinea pig ileum against the anaphylactoid effects of pathogenic staphylococcus bacterial extract or fraction A (F-10) (Fig. 1). This was demonstrated by the absence of initial contraction in the presence of serum or gamma globulin (criterion 1), and after washing out the serum or gamma globulin, by the typical anaphylactoid reaction, followed by tachyphylaxis, when a second and a third dose of extract or fraction A were added to the bath (criterion 2). Of forty-nine experiments with whole serum or gamma globulin from human, horse, rabbit, guinea pig, and bovine sources, protection was demonstrated in forty-one. Human serum was employed in ten instances, human gamma globulin in fifteen, horse serum in seven, rabbit serum in eleven, guinea pig serum in three, and bovine gamma globulin in three. Protection was demonstrated with all of these.

The heat stability of the protective effect was tested on seven ileum segments from seven guinea pigs by challenge with whole bacterial extract in three instances and with toxic fraction A (F-10) in four, following the initial addition of pooled human gamma globulin, previously heated at 56 degrees for thirty minutes. In all seven cases protection was unaffected, being present to the same degree as in the unheated gamma globulin.

In only one of the forty-nine experiments did serum or gamma globulin from the species mentioned fail to protect against the anaphylactoid reaction

*These fractions were kindly supplied by the Armour Laboratories.

†Kindly supplied by Pitman-Moore Company.

‡Kindly supplied by Dr. Leonhard Korngold, Sloan Kettering Institute for Cancer Research.

of bacterial extract or toxic fraction A (F-10). In that instance, normal rabbit serum was incubated with toxic fraction A at 37 degrees for thirty minutes. Addition of this mixture to the bath resulted in typical anaphylactoid contraction. After washing, addition of toxic fraction A caused no contraction, demonstrating tachyphylaxis. In five other experiments with five ileum segments from five guinea pigs, normal rabbit serum protected the guinea pig ileum against the bacterial extract or toxic fraction A.

In seven of the forty-nine experiments, the degree of protection was regarded as "undetermined," because the required criteria for protection were not fulfilled. Five of the seven showed slight or no reaction to the toxic bacterial extract or fraction A, when given with or immediately after serum, fulfilling the first criterion for protection. After washing out the perfusion bath, however, addition of extract or fraction A again resulted in slight or no reaction, therefore failing to fulfill the second criterion for protection. This may

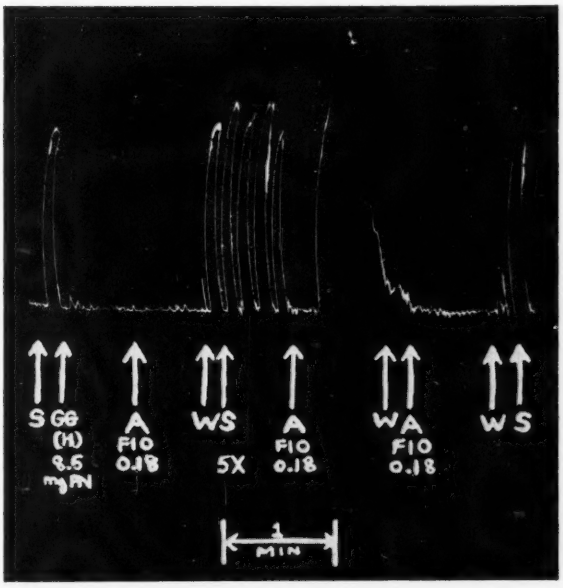

Fig. 1.—Protection by gamma globulin against the anaphylactoid effect of *Staphylococcus aureus* (F-10) fraction A upon isolated suspended segment of normal guinea pig ileum. *S* indicates addition of 0.1 mcg. of standard histamine base, followed by three washes at height of contraction. *W* indicates three washes.

At *G.G.* (*H*), 8.5 mg. protein nitrogen of pooled human gamma globulin added to bath. After one minute, addition of 0.18 mg. protein nitrogen of fraction A (F-10) fails to cause the customary contraction (first criterion for designation of "protection").

After five additions of histamine standard, with three washes between each, two additions of fraction A (F-10), separated by three washes, demonstrate the customary contraction with the first addition and lack of response (tachyphylaxis) with the second (second criterion for designation of "protection").

have resulted from the production of tachyphylaxis without a preceding contraction, or it may have been due to a segment of ileum which was unresponsive to toxic fraction A. Such a reaction could also result from incomplete rinsing of the bath, allowing a sufficient amount of serum to remain behind to protect against the second dose of fraction A.

Of the latter five cases of "undetermined" protection, two involved normal rabbit serum, one involved horse serum, and two involved human serum. However, five other tests with normal rabbit serum, three with Coombs' rabbit anti-gamma globulin, six with normal horse serum, and twenty-three with human serum or pooled human gamma globulin all showed protection against the toxic fraction A.

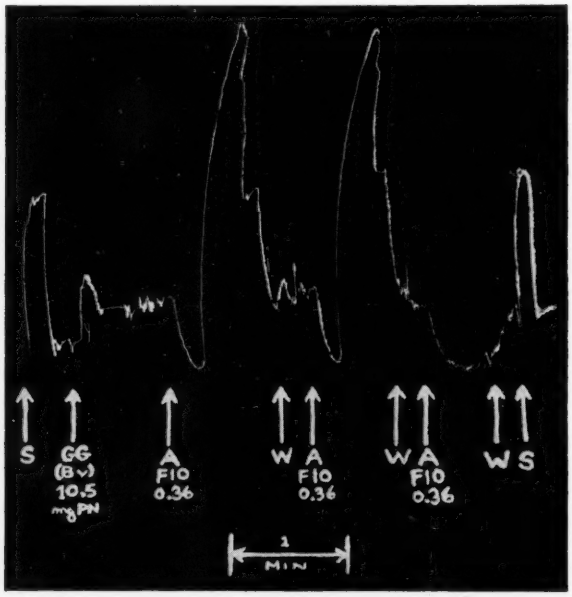

Fig. 2.—"Indeterminate protection" by gamma globulin against anaphylactoid effect of *Staphylococcus aureus* (F-10) fraction A upon isolated suspended segment of guinea pig ileum. *S* and *W* as in Fig. 1.

At *G.G.* (*BV*), 10.5 mg. protein nitrogen of pooled bovine gamma globulin added to bath, followed in one minute by addition of 0.36 mg. protein nitrogen of fraction A. Maximal contraction in fifteen seconds, followed by relaxation in thirty seconds.

After three washes at *A* (F-10), 0.36 mg. protein nitrogen of fraction A (F-10) again added. Maximum contraction, as before (that is, no tachyphylaxis).

After three washes, addition of same dose of fraction A as above results in no reaction (that is, tachyphylaxis).

Finally, two experiments with bovine gamma globulin gave undetermined protection against toxic fraction A (Fig. 2), which differed in type from the above five examples of "undetermined" protection. These utilized adjacent segments of ileum from a guinea pig which had been sensitized by four daily intradermal injections of 0.5 ml. of a 20 per cent solution of egg white four to five weeks prior to the experiment. In both instances there was a marked contraction to the initial dose of toxic fraction A, added while bovine gamma globulin was in the perfusion bath, indicating that there had been no protection. However, after thorough washing, maximal contraction followed a second dose of fraction A, free of bovine gamma globulin. A third addition of fraction A in both experiments resulted in no contraction, demonstrating

tachyphylaxis. It is of interest that protection by gamma globulin was demonstrated with a third segment of ileum from the same guinea pig and with five segments from other guinea pigs sensitized to egg white, not included in the present data.

With the exception of bovine beta globulin, no other materials studied protect the ileum against the anaphylactoid effects of fraction A (F-10). Serum albumin (bovine), fibrinogen (bovine), and alpha (human and bovine) and beta (human) globulin all failed to confer protection against the toxic fraction A, indicating that the protective effect is more or less limited to gamma globulin.

In three experiments, bovine beta globulin protected against bacterial extract (F-10) in one and showed indeterminate protection in two. No protection was demonstrated in two experiments with human beta globulin.

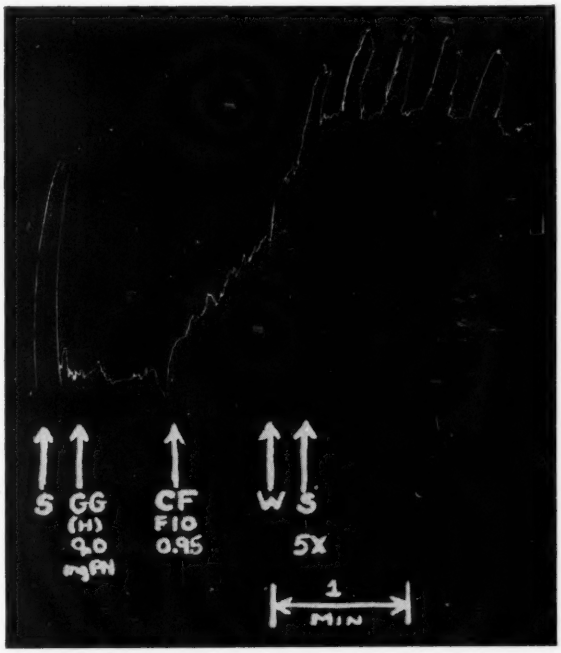

Fig. 3.—Absence of protection by pooled human gamma globulin (*G.G.* [*H*] 9.0 mg. of protein nitrogen) against myotonic effect of *Staphylococcus aureus* (F-10) culture filtrate (*CF* F-10 0.95 mg. protein nitrogen) upon isolated suspended segment of normal guinea pig ileum. *S* and *W* as in Fig. 1.

Studies of the protective effect of rat serum were carried out on eleven segments of ileum from six guinea pigs. In seven there was no protection, while four were indeterminate. Absence of protection was found with doses of rat serum as high as 0.5 ml., more than twice the amount sufficient for protection when sera from the other species were used.

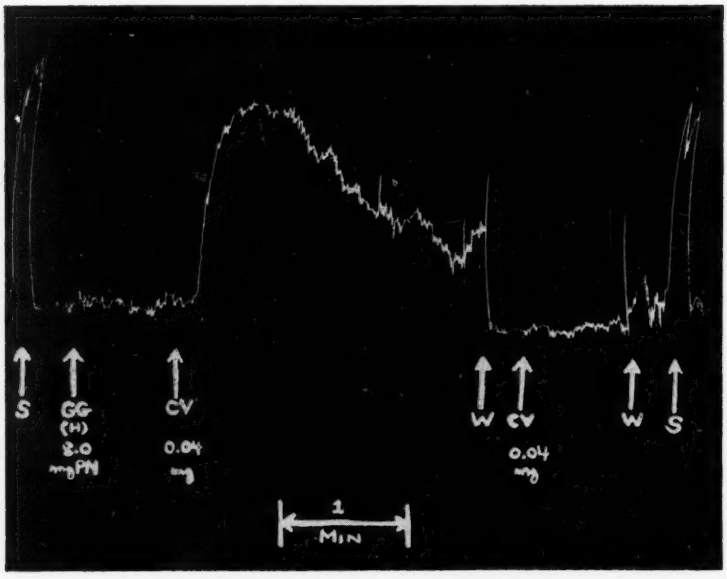

Fig. 4.—Absence of protection by pooled human gamma globulin (*G.G.* [*H*] 8.0 mg. protein nitrogen) against the anaphylactoid effect of cobra venom (*CV* 0.04 mg. of dried venom) upon isolated suspended segment of normal guinea pig ileum.

Note absence of response to second addition of venom (tachyphylaxis).

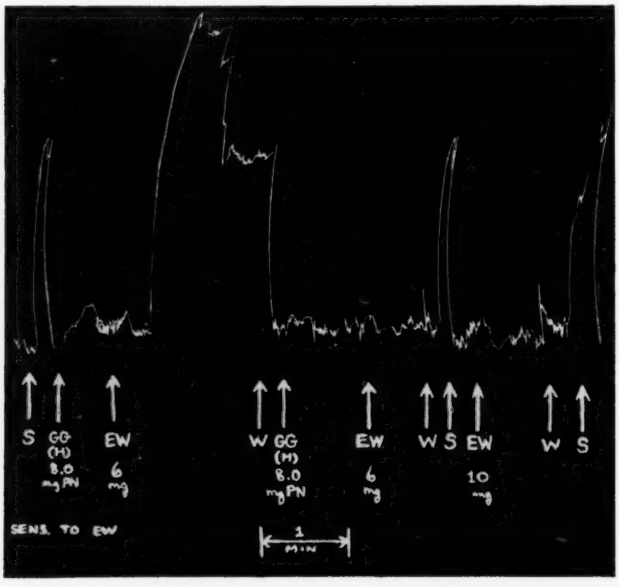

Fig. 5.—Absence of protection by pooled human gamma globulin (*G.G.* [*H*] 8.0 mg. protein nitrogen) against anaphylaxis in isolated suspended segment of ileum from guinea pig previously sensitized to egg white.

Note initial maximum anaphylactic response to 6.0 mg. of egg white (*EW*) and lack of response (desensitization) to second addition of egg white in presence of gamma globulin. Desensitization is complete in the presence of gamma globulin, since there is no response to 10.0 mg. of egg white after the gamma globulin is removed.

Twenty-five per cent solutions of glycerin, egg white, and egg yolk had no protective effect. Cobra venom was used in sufficient dosage to cause an anaphylactoid contraction and, following the demonstration of tachyphylaxis to venom, the ileum still reacted typically to staphylococcal bacterial extract or fraction A (F-10).

Neither serum nor gamma globulin conferred protection against the myotonic effects on the guinea pig ileum of staphylococcal culture filtrate (Fig. 3), cobra venom (Fig. 4), or histamine.

It was also found that neither serum nor gamma globulin had any noticeable effect upon anaphylaxis in the isolated guinea pig ileum from animals sensitized to egg white (Fig. 5), streptococcal proteinase, or two protein fractions derived from a nonpathogenic strain (F-7) of staphylococcus isolated in our laboratory and prepared as previously reported.[1, 3]

DISCUSSION

We have found that serum of several animal species protects the isolated guinea pig ileum against the anaphylactoid reaction regularly produced by a nucleoprotein fraction derived from washed, ground *Staphylococcus aureus* organisms. The protective effect is confined to the gamma globulin fraction. It is present in sera from all species thus far tested except the rat. The protective substance is stable at 56 degrees for thirty minutes. Its absence in rat serum apparently eliminates the properdin system from consideration as a factor in this phenomenon.[4]

The protection is specifically directed against the anaphylactoid effects of fraction A. Serum or gamma globulin has no effect upon other myotonic agents similarly studied, such as hemolytic staphylococcal culture filtrate, cobra venom, or histamine.

Of particular interest are the two instances of "indeterminate protection" (Fig. 2), in which gamma globulin failed to protect the segment of guinea pig ileum against the toxic fraction A, but in which, after washing, a second and third addition of fraction A without gamma globulin resulted in a typical anaphylactoid reaction with tachyphylaxis. If there had been no protection, one would have expected the ileum to be refractory to subsequent additions of fraction A. This could represent a situation in which only partial protection was conferred by the gamma globulin with incomplete tachyphylaxis, and therefore subsequent addition of fraction A caused a contraction. Another possible explanation is that the ileum was from an animal which had become hypersensitive, through natural means, to fraction A or a closely related protein. If this were so, the contraction of the ileum when fraction A was added in the presence of gamma globulin would represent an anaphylactic reaction, the gamma globulin protecting against the toxic effect of the fraction A, but allowing the anaphylactic reaction to occur. Subsequent addition of fraction A in the absence of gamma globulin would represent the toxic effect of fraction A. This explanation correlates with our findings that gamma globulin did not protect against anaphylaxis in the isolated ileum. It also fits in with the fact that the two segments of ileum that reacted in this way were adjacent

segments from the same animal. The third adjacent segment did not so react, but we have found, in confirmation of Kempf and Feinberg,[5] that if adjacent segments of ileum from sensitized animals are tested, some nonreactive segments occasionally can be found. The fact that the guinea pig from which these two segments of ileum demonstrated this unusual type of "indeterminate" protection had been made hypersensitive to egg white by prior injections is probably unrelated to the phenomenon, since segments of ileum from other guinea pigs, similarly sensitized to egg white, reacted no differently from segments from normal animals.

If these two instances of "indeterminate" protection are due to hypersensitivity to fraction A, as postulated, it should be possible to induce and demonstrate anaphylaxis to this fraction, despite its primary toxicity, by prior addition to the bath of gamma globulin, which would protect the ileum segment against the toxic effect of fraction A while permitting the anaphylactic reaction to occur. Such studies are in progress.

CONCLUSION

A heat-stable factor in human, horse, rabbit, bovine, and guinea pig serum protects the isolated guinea pig ileum against the anaphylactoid effects of a toxic nucleoprotein fraction derived from washed, ground *Staphylococcus aureus* organisms. Rat serum was the only serum studied that failed to protect. The protective effect is found almost exclusively in the gamma globulin fraction, and possibly to some extent in the beta globulin fraction.

The protective effect is specific, since it is not directed against certain other myotonic agents, such as hemolytic staphylococcal culture filtrate, cobra venom, or histamine. Neither whole serum nor gamma globulin protects against anaphylaxis in the isolated guinea pig ileum.

It is suggested that gamma globulin, by protecting the ileum against the anaphylactoid effect of the nucleoprotein fraction and allowing anaphylaxis to occur, may make it possible to demonstrate anaphylaxis in animals rendered hypersensitive to this primarily toxic fraction.

REFERENCES

1. Dworetzky, M., Zeitlin, B. R., Kahn, M. C., and Baldwin, H. S.: Pharmacologic and Anaphylactogenic Properties of Staphylococcus Protein and Unfractionated Extract, J. ALLERGY **23**: 359-364, 1952.
2. Dworetzky, M., Baldwin, H. S., and Smart, K. M.: Biologic Effects of Materials Derived From Staphylococcus: Further Studies, J. ALLERGY **27**: 39-49, 1956.
3. Kahn, M. C., Baldwin, H. S., Zeitlin, B. R., and Smart, K. M.: Cutaneous Reactions to Staphylococcus Polysaccharide, Protein, and an Unfractionated Extract in Hypersensitive and Normal Individuals, J. ALLERGY **22**: 237-248, 1951.
4. Pillemer, L., Shoenberg, M. D., Blum, L., and Wurz, L.: Properdin System and Immunity. II. Interaction of the Properdin System With Polysaccharides, Science **122**: 545-549, 1955.
5. Kempf, A. H., and Feinberg, S. M.: Dissimilar Sensitivity to Antigen of Segments From Isolated Intestinal Strip, J. ALLERGY **19**: 247-250, 1948.

MEPHENESIN (TOLSEROL) SENSITIVITY WITH DEMONSTRABLE SKIN-SENSITIZING ANTIBODY IN THE SERUM

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ATTEMPTS to demonstrate a whealing type of skin reaction or the presence of skin-sensitizing antibodies in the serum of patients who have had an allergic reaction to drugs of a simple chemical nature have been unsuccessful for the most part. Sherman and Cooke[1] obtained positive passive transfer reactions to sulfadiazine and sulfapyridine in sites sensitized with the serum of a patient who had experienced a severe reaction to sulfadiazine. Ingestion of sulfadiazine resulted in reactions of passively sensitized sites in forty to ninety minutes.

Recently we have seen a patient with a severe immediate reaction to Tolserol (mephenesin) and we have been able, by passive tranfer, to demonstrate skin-sensitizing antibodies to this drug.

Tolserol†[2, 3] (3-ortho-toloxy-1,2-propanediol) is a central nervous system depressant used in anxiety states and neuromuscular disorders. It has its main site of action on the midbrain, brain stem, at the level of the thalamus, and in the spinal cord probably at the internuncial neurons. The drug is rapidly absorbed when given orally and is detoxified by the liver as B (orthotoloxy) lactic acid. It exerts muscle-relaxing properties with little effect on the blood pressure or respiration. Untoward reactions described include circumoral anesthesia, flushing of the face, nausea, vomiting, dizziness, diplopia, blurring of vision, pruritus, tinnitus, and horizontal and vertical nystagmus. Intravascular hemolysis, hemoglobinuria, and fatal anuria have occurred. Red cell casts and protein in the urine following the use of the drug have been reported in the literature.[3] We were unable to find any reports of an acute anaphylactoid reaction to mephenesin.

CASE REPORT

A 41-year-old white married woman was admitted to Milwaukee County General Hospital for the first time on July 24, 1956, following an acute drug reaction. She had been seen that morning in the outpatient department because of left-sided neck pain of two weeks' duration. Examination revealed moderate spasm with tenderness of the left sternocleidomastoid muscle, as well as an edematous, injected pharynx. Tablets of aspirin-phenacetin-caffeine (APC), Tolserol, and tetracycline were prescribed. After she reached

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†Trademark of E. R. Squibb & Sons, New York, N. Y.

home, she took one of each of these medications. Approximately twenty minutes later, she experienced diaphoresis and circumoral and upper extremity numbness, with tingling and tightness of the chest. She was taken immediately to Johnston Emergency Hospital where she received supportive therapy, with a prompt recovery. She then was transferred to the medical service at the Milwaukee County General Hospital.

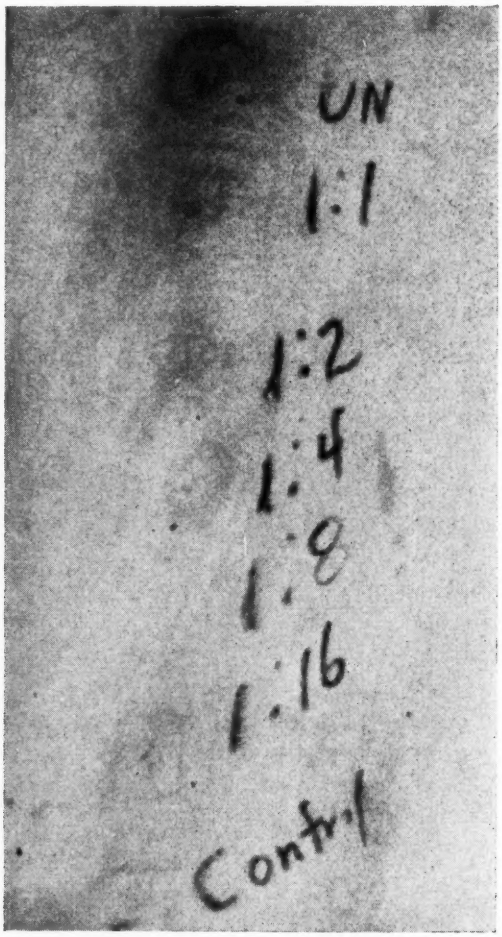

Fig. 1.—Whealing reaction in passively sensitized skin sites (subject A) thirty minutes after ingestion of 500 mg. mephenesin (Tolserol). An appreciable reaction occurred through a 1:16 serum dilution.

In 1952 she had had a hysterectomy because of a fibroid tumor of the uterus. Chronic cholecystitis with cholelithiasis was also diagnosed at that time. There was no history of any previous drug reactions or allergic disorders.

The physical examination revealed a well-developed, well-nourished, somewhat apprehensive white woman who did not appear acutely ill. Blood pressure was 120/80; pulse, 80; respirations, 18. The head showed no abnormalities. Minimal spasm and tenderness of the left sternocleidomastoid muscle was noted. Examination of the heart, lungs, abdomen, pelvis, rectum, extremities, skin, reflexes, and sensorium showed no abnormalities.

TABLE I. PASSIVE TRANSFER REACTIONS OBTAINED WITH SERUM TAKEN ON DAY OF SECOND REACTION

DILUTION OF SERUM	FIRST-DAY SERUM TEST SUBJECTS			
	A	B	C	D
Undiluted	4+	3+	0	3+
1:1	3+	2+	0	0
1:4	2+	1+	0	0
1:8	1+	0	0	0
1:16	1+	0	0	0
Control	0	0	0	0

Chest x-ray was normal. The hemoglobin was 13 grams; white blood count was 11,700, with a differential of 13 stabs, 51 segmented polymorphonuclears, 30 lymphocytes, 5 monocytes, 1 basophil and no eosinophils. A Wassermann test was negative. The urine, blood, nonprotein nitrogen, and fasting blood sugar were all normal. Routine cutaneous and intracutaneous tests with inhalants and food allergens were negative.

The resident on the service believed clinical trial by readministration of the drugs in question to be justified. Accordingly, one APC tablet was given on the patient's second hospital day, and one capsule of tetracycline was given on the third day without reaction. On the fourth hospital day, she was given 250 mg. of Tolserol orally. Within thirty minutes, circumoral angioedema was apparent and she complained of numbness and tingling of the upper extremities and tightness of the chest. At this time, some inspiratory and expiratory wheezing was audible. There was a precipitous fall in blood pressure from 110/70 mm. to 50 mm. systolic pressure and this time her diastolic pressure could not be determined. She was given 50 mg. of Benadryl intravenously at once, and intravenous administration of one ampule of l-Arterenol and 100 mg. of hydrocortisone diluted in one liter of 5 per cent glucose in water was started also. Within a few minutes, the angioedema had lessened markedly. One hour later all symptoms had disappeared and only a few urticarial lesions remained. A direct cutaneous test with 1 per cent aqueous mephenesin solution was negative.

Twenty cubic centimeters of blood was drawn at this time and sterile serum was prepared. Ring precipitin tests with this serum and a 10 per cent ethyl alcohol solution of Tolserol were negative. Additional samples of serum were obtained from the patient after intervals of ten and thirty days. Each serum was serially diluted with normal saline. Skin sites were prepared on the backs of four nonsensitive persons by intradermal injections of 0.1 ml. of undiluted serum and 1:1, 1:4, 1:8, and 1:16 dilutions. Control sites were prepared with normal serum from both A and B type bloods. Forty-eight hours later, 500 mg. of Tolserol was given orally to each of four sensitized subjects.

TABLE II. PASSIVE TRANSFER REACTIONS OBTAINED WITH SERA TAKEN AFTER RECOVERY

DILUTION OF SERUM	TEN-DAY SERUM TEST SUBJECTS		THIRTY-DAY SERUM TEST SUBJECTS	
	A	E	F	G
Undiluted	+	0	+	0
1:1	0	0	0	0
Control	0	0	0	0

A marked whealing reaction occurred within ninety minutes after ingestion of the drug in sites sensitized with undiluted serum in three of the four test subjects. In two subjects the reaction was apparent within thirty minutes. In one subject (A), an appreciable reaction occurred in sites sensitized with serum through a 1:16 dilution (Fig. 1); another subject (B) showed reactions through a 1:4 dilution; another (D) had a detectable reaction only in the undiluted serum site. One subject, a large, obese man (C), had

no detectable reaction in any site. Serum obtained ten days after the initial drug reaction was used to sensitize sites in two subjects, one of whom (A) had shown reactions after ingestion of Tolserol in the first experiment. A positive reaction was again obtained in subject A in the undiluted serum site; no reaction was detected in the other subject (E). One of two additional subjects sensitized with undiluted serum obtained thirty days after the initial drug reaction reacted in the site sensitized with undiluted serum after ingestion of the test dose of Tolserol (Table II).

SUMMARY

Skin sites were sensitized in normal recipients with serum obtained from a patient immediately after an allergic reaction to ingested Tolserol. Positive reactions occurred within thirty to ninety minutes in the sensitized skin sites of three of the four subjects tested by ingestion of a challenging dose of 500 mg. of Tolserol. Some skin-sensitizing activity could still be demonstrated in serum withdrawn thirty days after the patient's reaction to Tolserol.

REFERENCES

1. Sherman, W. B., and Cooke, R. A.: Sulfadiazine Sensitivity With Demonstrable Skin-Sensitizing Antibody in the Serum, Am. J. Med. **2**: 588, 1947.
2. Beckman, H.: Pharmacology in Clinical Practice, Philadelphia, 1952, W. B. Saunders Company.
3. Clendon, D., and Penfold, J.: Hemolytic Effects of Myanesin, Lancet **2**: 987, 1949.

THE RISK OF INDUCING CONSTITUTIONAL REACTIONS IN ALLERGIC PATIENTS

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ADEQUATE management of the allergic person involves the use of procedures which occasionally produce undesirable systemic reactions. Forty years ago Cooke and Vander Veer[1] reported the incidence of reactions to hyposensitization injections to be about 3.5 per cent; more recent surveys have shown this incidence to be somewhat lower, and have indicated precautions which should be taken to keep the number of reactions at a minimum.[2-8]

On the other hand, we have little information concerning the frequency of untoward effects from skin testing. In one of Cooke's[9] earlier studies, about 2.5 per cent of patients given intradermal tests had immediate constitutional reactions. In Peshkin and Fineman's[10] study of 100 children, reported twenty years ago, 2 per cent had general reactions following intradermal testing; none had trouble following scratch tests. Since then, the only published information was that reported by Courtright[11] in 1943, in which three of 300 patients developed systemic reactions, none fatal, following intradermal testing. In this group, scratch testing was usually done first.

Much more emphasis has been placed on the isolated, very severe, or fatal reactions from intradermal testing. In 1949, Blanton and Sutphin[12] reported one death which followed multiple intracutaneous tests, and cited the ten previous fatalities reported from 1922 to 1946. A year later, Harris and Shure[13] reported a death from intradermal testing to cottonseed, and cited two other deaths from the same cause. A brief perusal of the literature reveals casual mention of other deaths from intradermal skin testing;[14, 15] presumably a small number of fatalities have never been recorded. No documented reports on deaths from scratch testing have appeared, although two deaths are mentioned briefly by Vaughan and Black.[16]

A study of the reported fatalities, plus several of the severe near-fatal reactions from skin testing which have been reported,[17-22] indicates that the reactions usually occurred when the substances were given in amounts which would never be used in present-day intradermal testing. For example, fatalities occurred from the use of 0.1 c.c. of undiluted egg white,[23] a 1:500 solution of buckwheat,[24] and 0.05 c.c. of undiluted horse serum.[25] Other fatalities

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or severe reactions have followed the use of unfamiliar substances, such as guinea pig hemoglobin[26] and asthma powders,[19] or substances of high sensitizing potential, such as castor bean,[27] buckwheat,[16, 17] mustard,[18] and cottonseed.[13, 20] It should be noted that some of these severe reactions occurred from scratch testing alone.

Two of the four fatal reactions which have been reported during the last decade occurred after the administration of more than fifty intradermal tests, in spite of the prior use of scratch tests.[12, 28]

In order to evaluate the incidence of constitutional reactions from treatment in recent years, and to obtain an accurate impression of the relative importance of reactions from intradermal skin testing in a large series, we felt it worth while to review the records of 8,706 patients with allergic symptoms seen from 1935 to 1955 in the Allergy Clinic of Roosevelt Hospital.

CLINICAL MATERIAL

A majority of the patients studied had either asthma or hay fever. Those with atopic dermatitis, urticaria, or eye symptoms were also included if significant skin sensitivity existed. Patients with contact dermatitis, emphysema, respiratory infections, and other nonallergic or questionably allergic states were not included. Almost all the patients in this series received hyposensitization treatments; those who received no injections at this clinic were not included unless a constitutional reaction had occurred on skin testing.

Table I presents the diagnoses in this group of patients. These patients received a total of approximately 1,250,000 treatment injections over the twenty-one-year period under consideration.

TABLE I. PATIENTS SEEN AT ROOSEVELT HOSPITAL INSTITUTE OF ALLERGY, 1935-1955

DIAGNOSIS			SENSITIVITY		
Asthma (alone)			Pollen (usually ragweed)	4,215	48.4%
Pollen	76				
Dust (usually with other inhalants)	1,587		Dust	2,699	31.0%
Other	386				
Negative tests	425		Other skin sensitivity	946	10.9%
Total		2,474	Negative tests	846	9.7%
Hay fever (pollen-sensitive)	3,476				
Pollen season, negative tests	38				
Total		3,514			
Asthma and Hay fever (pollen-sensitive)		663			
Rhinitis					
Dust-sensitive	956				
Nonseasonal, positive tests	277				
Negative tests	356				
Total		1,589			
Asthma and Rhinitis					
Positive skin tests	156				
Negative tests	27				
Total		183			
Atopic dermatitis, urticaria, or eye symptoms without respiratory symptoms		283			
Grand Total		8,706			

TABLE II. STRENGTH OF MATERIALS ROUTINELY USED FOR INTRADERMAL TESTING AT ROOSEVELT HOSPITAL

SUBSTANCE	SOLUTION	SUBSTANCE	SOLUTION
Dust	Concentrated	Gums	1%
Pollens	100 P.N.U./c.c. each	Most foods	1,000 P.N.U./c.c. each
Molds	1,000 P.N.U./c.c. each	Milk	10,000 P.N.U./c.c.
Feathers	1,000 P.N.U./c.c.	Egg and peanut	100 P.N.U./c.c. each
Kapok	100 P.N.U./c.c.	Mustard	10 P.N.U./c.c.
Epidermals	100 P.N.U./c.c. each	Cottonseed	10 P.N.U./c.c.
Pyrethrum	1,000 P.N.U./c.c.		

Intradermal skin testing was performed by physicians familiar with the patient's history, in a location on the arm permitting effective use of a tourniquet. Testing to a group of six substances was ordinarily followed by five to ten minutes of observation before further testing was performed. The concentration of material used depended on its sensitizing potential. If the clinical history indicated marked sensitivity, more dilute solutions were ordinarily used for testing in the case of pollens, and skin testing was usually avoided in the case of such foods as shellfish and nuts. Certain substances were tested with solutions more dilute than those usually employed; these were cottonseed, egg, mustard, and peanut (Table II). Furthermore, testing was arranged so that each group of six tests contained substances as different as possible from

TABLE III. CRITERIA USED FOR GRADING CONSTITUTIONAL REACTIONS

1. *Slight and Questionable* (Grade I)
   (a) Mild symptoms of "hay fever," especially during pollen season, or moderate hay fever symptoms starting more than four hours after injection
   (b) Tingling or itching in palms without other symptoms, subsiding without treatment
   (c) Transient, delayed urticaria; no treatment
2. *Mild* (Grade II)
   (a) Urticaria alone, treated or untreated
   (b) "Hay fever," treated or untreated
   (c) Both (a) and (b), not severe
   (d) Coughing or choking alone, or with (a) and (b), untreated
   (e) Flush and redness of face, alone
   (f) Undescribed "constitutional" requiring no treatment
3. *Moderate* (Grade III)
   (a) Immediate marked hay fever with flush, coughing, and/or urticaria necessitating treatment
   (b) Abdominal cramps alone or with (a)
   (c) Some shortness of breath and/or wheezing
   (d) Undescribed "constitutional" requiring routine treatment, such as epinephrine or antihistamine, tourniquet
4. *Moderately Severe* (Grade IV)
   (a) Acute combination of symptoms (hay fever, urticaria, itching, flush) getting rapidly worse, stopped by treatment
   (b) Abdominal or uterine cramps, alone, when incapacitating to patient
   (c) Active asthma, with or without slight cyanosis
   (d) Undescribed "constitutional" (rare) in which repeat epinephrine treatment was necessary
5. *Severe* (Grade V)
   (a) Any of above symptoms with cyanosis and/or shock
   (b) Above symptoms, necessitating more prolonged treatment, including oxygen, hospitalization

other groups, in order to reduce the possibility of multiple marked reactions. Scratch testing was used only for occasional preliminary evaluation of substances of unknown sensitizing ability.

TABLE IV. SEVERITY AND CAUSE OF REACTIONS FROM TREATMENT

SUBSTANCE	GRADE I	II	III	IV	V	TOTAL
Ragweed	67	171	206	59	10	513
Ragweed and other	5	54	41	24	2	126
Grass	1	12	7	1	0	21
Grass and other	0	3	1	2	0	6
Trees	0	1	1	0	0	2
Plantain	0	1	1	0	0	2
					Total pollen	670*
Stock dust	1	2	4	0	0	7
Autogenous dust	0	0	1	0	0	1
Autogenous vaccine	0	0	2	0	0	2
Molds	1	0	2	2	0	5
Cottonseed	0	1	2	2	2	7
Misc. (cat feathers)	1	3	2	0	0	6
Misc. (kapok)	76	248	270	90	14	28
Misc. (dog)						670
					Grand Total	698*

*Several patients had reactions from different substances at different times; this figure includes these duplications.

RESULTS

A total of 1,774 constitutional reactions occurred from the estimated 1,250,000 treatment injections; about one in 700. However, these reactions occurred in only 663 patients; in these persons, then, the incidence was 1.9 per cent, or one in fifty-four injections. Of these, 635 were pollen sensitive.

TABLE V. PREDOMINANT SYMPTOMS OR SIGNS IN 1,774 CONSTITUTIONAL REACTIONS FROM TREATMENT

SYMPTOMS	FREQUENCY (PER CENT)	OTHERS	NUMBER OF CASES
Hay fever-like	18.3	Headache	12
Asthma, wheezing, or tightness in chest	16.2	Apprehension and tachycardia	8
Urticaria and/or angioneurotic edema	14.5	Shock (not syncope)	6
Itching of extremities	9.3	Diarrhea	3
Choking and/or coughing	9.3	Uterine cramps	3
Combinations	7.3	Dizziness	2
Flush only	2.6	Fullness in ears	2
Abdominal cramps	1.1	Cyanosis	1
Headache	1.1	Chills	1
Other (see next column)	2.2		38 = 2.2%
No description	18.1		

Minor or Associated Symptoms Not Noted Above:

Numbness in mouth	Weakness
Cold sweat	Blurred vision
Trembling	"Tingling"
Premature menstrual period	"Bad feeling"
Fullness in head	Transient chest pain

Accordingly, 15 per cent of the total group of pollen-sensitive patients had one or more constitutional reactions. Among the remaining 4,491 patients, only twenty-eight suffered any generalized reactions; an incidence of 0.6 per cent. Those with negative skin tests who were included in this study were given stock or autogenous bacterial vaccines; they are included in the above figures.

The constitutional reactions were gauged as to severity according to the criteria outlined in Table III. These data are presented in Table IV, in terms

TABLE VI. POSSIBLE CAUSES OF CONSTITUTIONAL REACTIONS TO TREATMENT

POSSIBLE CAUSE			NUMBER OF CASES
A. Failure to reduce treatment dose adequately during pollen season			
1. Dose maintained	97		
2. Dose actually increased	84		
3. Dose decreased to 75-90%	16		
4. Dose decreased to 50-74%	6		
5. Dose decreased to < 50%	0		
Total			203
B. Limit of patient's tolerance			
*P.N.U.**	*P.N.U.*	*P.N.U.*	
5—1	1,000—20	15,000—1	
100—23	2,500—32	20,000—1	
500—60	5,000—15		
Total			153
C. Too Great an Increase in Dosage			
Increase			
200%— 1			
85—100%—70			
51— 85%—17			
Total			88
D. Cumulative Effect of Maintenance Doses			
1. Large doses (> 4,000 P.N.U.) given at 2- or 3-week intervals	18		
2. Maintenance doses continued unreduced after end of season	41		
Total			59
E. Interval between maintenance injections longer than 4 weeks			33
F. Failure to reduce dose sufficiently when changing to new extract			
1. Dose not reduced	5		
2. Dose reduced to 50-75%	7		
3. Dose reduced to 40-49%	2		
4. Dose reduced to < 40%	0		
Total			14
G. Performance of skin tests along with treatment at low doses			7
H. High concentration by error, at clinic			6
I. Apparent intolerance following one constitutional reaction			5
J. Addition of new antigen to injection regimen			5
K. Possible intravenous injection of extract			4
L. High concentration by error, doses furnished by clinic but given elsewhere			3
M. Wrong pollen by error			1
			581
N. Unexplained			1,193
		Total	1,774

*Protein nitrogen units.

of the number of patients involved rather than the total number of constitutional reactions observed. Each patient is classified according to the most severe reaction which he had suffered at any time.

The predominating symptoms observed in the 1,774 constitutional reactions showed the distribution outlined in Table V. When clear-cut combinations of symptoms occurred (such as asthma-urticaria, hay fever with coughing and choking, or asthma and abdominal cramps), they were tabulated separately. Unfortunately, in a large number of cases, adequate description of the symptoms of the particular reaction was lacking.

TABLE VII. NUMBER OF REACTIONS FROM TREATMENT PER PATIENT

REACTIONS	NO. OF CASES
1	312
2- 4	255
5- 9	76
10-15	16
17	2
20	1
21	1
	663

The possible or probable cause of these reactions was investigated from the information recorded in the case records. Although these data were far from adequate, certain patterns were apparent and are presented in Table VI. The number of cases in which reactions were only coincidentally related to the proposed cause would be impossible to estimate. Because of the nature of the sensitivity distribution of the 663 patients who had systemic reactions, most of our material represented reactions to treatment with pollen extracts. Some of these patients had multiple reactions in spite of the usual precautions (Table VII).

TABLE VIII. SEVERITY OF REACTIONS TO INTRADERMAL TESTING

TEST SUBSTANCE	GRADE				TOTAL
	I	II	III	IV	
Ragweed	8	8	7	1	24
Ragweed and grass		2	2		4
Trees	1	3			4
Plantain		1			1
Mugwort, Marsh elder		1			1
Grass, Plantain, birch			1		1
Cosmos and dahlia			1		1
Several inhalants	1		1		2
Tomato and pork				1	1
Unknown food	1				1
Cottonseed	1	1			2
				Total	42

Only a few reactions were noted from intradermal skin testing alone, and these are indicated in Table VIII. Criteria used to grade the reactions were

those outlined in Table III. Except for cottonseed, routine testing to substances of high sensitizing potential was associated with no difficulty, although significantly positive skin tests to these materials occurred on many occasions (Table IX).

TABLE IX. NUMBER OF PATIENTS WITH +++ OR ++++ SKIN REACTIONS ON ROUTINE TESTING WITH HIGHLY SENSITIZING SUBSTANCES*

SUBSTANCE	NO. OF PATIENTS	SUBSTANCE	NO. OF PATIENTS
Shellfish	248	Fish	47
Egg	106	Flaxseed	20
Cottonseed	96	Mustard	19
Walnut	59	Castor bean† (1 P.N.U./c.c.)	7
Peanut	54		

*Buckwheat is not used routinely.
†No longer used for routine testing.

Thirty-six persons had constitutional reactions from testing with pollen extracts. This is 0.85 per cent of the total pollen-sensitive group. In the remaining 3,645 patients with positive skin tests to other substances, only six reactions occurred (an incidence of 0.16 per cent). If the 2 per cent incidence of reactions from cottonseed is deleted from this latter group, the occurrence of reactions approaches one in 900. It is significant that no alarming reactions ever occurred; only two of Grade IV severity were noted.

TABLE X. CAUSES OF CONSTITUTIONAL REACTIONS FROM INTRADERMAL TESTING

1. Tests performed with greater than recommended concentrations*		25
2. Simultaneous testing with two or more pollens, all giving strong skin reactions	7	
Not included in (1)		3
3. Multiple testing with many marked reactions		1
4. Testing performed at same time as treatment injections	10	
Not included in (1) or (2)		6
5. History of extreme sensitivity to ragweed with previous constitutional reactions; routine concentration used		1
		36

*In many cases these reactions occurred when sensitivity titration was performed hastily by intradermal administration of three concentrations (usually 10, 100, and 1,000 P.N.U./c.c.) of pollen extract simultaneously.

Analysis of the circumstances which led to the reactions from testing was much more fruitful than in the study of the treatment group. Thirty-six of the forty-two reactions occurred when standard precautions were not used (Table X). One additional reaction was due to the rarely used pollen extracts, cosmos and dahlia, which were not standardized by chemical analysis. Of the remaining five reactions, two were Grade I, two were Grade II, and one was Grade IV in severity. The last one followed simultaneous testing to nine foods, the only reactions being +++ to tomato and pork.

DISCUSSION

When pollinosis is treated aggressively by injection of allergen extracts, systemic reactions are unavoidable. Although only about 2 per cent of our injections were accompanied by reactions, one patient in seven experienced at least one generalized reaction, and some patients had repeated reactions but continued to return for what was considered to be effective treatment. Only one third of the reactions appeared to be preventable, even though many reactions which occurred under debatable circumstances were included in the "avoidable" group.

Determining the causes of constitutional reactions which occurred during the pollen season was particularly difficult. Many variables came into play at this time, not the least of which was the uncontrollable use of symptomatic medication.

The data in Table VI support in general the precautions previously cited,[2-6] although certain of the commonly recognized errors were noted only rarely in this series. One phenomenon which occurred rather frequently has not been stressed previously. This involved the appearance of reactions after the end of a pollen season, when maintenance doses were being continued for perennial treatment. This type of reaction was particularly remarkable in those patients who had no reactions to injections maintained at a high level during the pollen season; it also occurred in some persons for whom the dose had been reduced significantly at the onset of the season. Accordingly, it appeared worth while, when committing a patient to year-round treatment, to administer postseasonal injections cautiously, at relatively low doses.

Cottonseed caused particular trouble in the treatment group. Most of the low-tolerance cases cited in item B, Table VI, were associated with cottonseed administration. Desensitization to this substance was, of course, rarely successful, and was abandoned in most cases in this clinic several years ago.

It was difficult to evaluate limits of tolerance. A certain group of patients invariably suffered a systemic reaction when a particular dosage level had been reached. In other cases, the physician had assumed that the limit of tolerance was reached when one isolated reaction occurred; accordingly, that dose was never attained again. This type of precaution was apparently unnecessary, since most patients who had their first constitutional reaction at a certain dosage level were able, after a moderate reduction in amount, to tolerate much higher doses when increase was again attempted. The data in Table VI therefore include only those patients in whom two or more attempts at raising dosage above a certain "threshold" resulted in systemic reactions.

Reduction of dosage at certain stages seemed well worth while. In our experience, reactions could be avoided by diminishing dosage to less than 40 per cent when changing from an old to a fresh extract. Similarly, cutting dosage in half during the appropriate pollen season probably prevented some reactions.

In comparison to the treatment reactions, reactions to intradermal testing were relatively unimportant, both in frequency and in severity. The precautions taken in this clinic apparently were effective, since thirty-seven of the forty-two reactions which we recorded occurred when the recommended precautions were disregarded or had not been instituted.

Under adequate supervision, the danger of direct intracutaneous testing appeared to be very much less than the risks routinely taken in injection therapy of the same patients, and should be considered in such perspective (Fig. 1). Of course, this would remain true only as long as proper precautions were carefully observed. We would certainly agree that, if direct supervision of skin testing by an experienced person is not possible, the prior performance of scratch testing is the most efficient precaution now available. However,

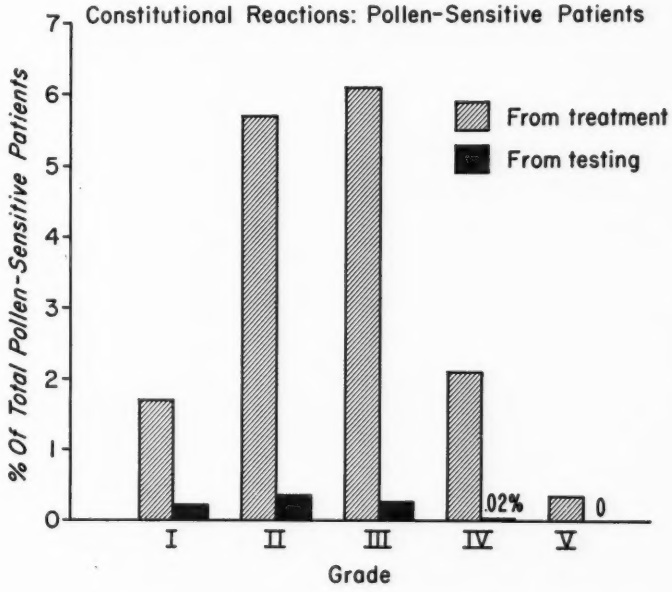

Fig. 1.—Incidence of constitutional reactions in pollen-sensitive patients. Comparison between testing and treatment reactions.

this procedure may lead to a false sense of security, as exemplified by the above-mentioned two recent fatal cases[12, 28] and by the recently reported near-fatal reaction from castor bean testing.[27]

It is apparent that the occurrence of constitutional reactions is inevitable when treatment dosages of allergenic extracts are steadily increased until clinical improvement is marked or until the patient's level of tolerance is exceeded. On the other hand, reactions to intradermal skin testing should be exceedingly rare, if the tests are selected carefully by reference to the patient's clinical history and if one avoids indiscriminate routine testing to an unnecessarily large number of substances.

SUMMARY

Case records of 8,706 allergic patients seen at Roosevelt Hospital Institute of Allergy were reviewed, and the type and frequency of constitutional reactions were noted.

A great majority of reactions occurred from the use of pollen extracts, both in testing and in treatment. There were 1,774 reactions from treatment; these occurred in 663 patients. A possible precipitating cause could be detected in only about one-third.

Skin testing was performed intradermally without prior use of scratch tests in all cases. The reactions from testing were infrequent and mild, compared to the reactions from treatment, and occurred in only forty-two patients. Thirty-seven of these reactions could have been avoided, had the usual precautions been followed.

No fatalities occurred in this group of patients following either treatment or testing injections.

REFERENCES

1. Cooke, R. A., and Vander Veer, A., Jr.: Human Sensitization, J. Immunol. **1:** 201, 1916.
2. Vander Veer, A., Jr., Cooke, R. A., and Spain, W. C.: Diagnosis and Treatment of Seasonal Hayfever, Am. J. M. Sc. **174:** 101, 1927.
3. Markow, H., and Spain, W. C.: Ragweed Hayfever, Its Diagnosis and Treatment, J. ALLERGY **1:** 414, 1930.
4. Waldbott, G. L.: The Prevention of Anaphylactic Shock, J. A. M. A. **98:** 446, 1932.
5. Furstenberg, F. F., and Gay, L. N.: The Occurrence of Constitutional Reactions in the Treatment of Hay Fever and Asthma: Analysis of the Causative Factors, Bull. Johns Hopkins Hosp. **60:** 412, 1937.
6. Greene, J. E.: Constitutional Reactions in Hayfever Therapy, M. Clin. North America **23:** 1255, 1939.
7. Sherman, W. B., and Hebald, S.: A Method of Determining the Probability of Constitutional Reactions During Treatment of the Ragweed Hay Fever Patient, Am. J. M. Sc. **203:** 383, 1942.
8. Zonis, J., and Rubin, N.: The Prediction of Constitutional Reactions by Testing With Ragweed Pollen Fractions, J. ALLERGY **18:** 36, 1947.
9. Cooke, R. A.: Studies on Specific Hypersensitiveness. III. On Constitutional Reactions: The Dangers of the Diagnostic Cutaneous Test and Therapeutic Injection of Allergens, J. Immunol. **7:** 119, 1922.
10. Peshkin, M. M., and Fineman, A. H.: Asthma in Children. VI. A Comparative Study of the Scratch and Intradermal Methods of Skin Testing, Am. J. Dis. Child. **34:** 815, 1927.
11. Courtright, L. J.: Critical Review of Intradermal Skin Tests as Used by Stanford Asthma Clinic, 1940-41, Stanford M. Bull. **1:** 120, 1943.
12. Blanton, W. B., and Sutphin, A. K.: Death During Skin Testing, Am. J. M. Sc. **217:** 169, 1949.
13. Harris, M. C., and Shure, N.: Sudden Death Due to Allergy Tests, J. ALLERGY **21:** 208, 1950.
14. Kahn, I. S. (p. 205), and Rowe, A. H. (p. 207): Discussions Following Rowe, A. H.: An Evaluation of Skin Reactions in Food-Sensitive Patients, J. ALLERGY **5:** 135, 1934.
15. Sheldon, J. M., Mathews, K. P., and Lovell, R. G.: Skin Tests in Atopic Disease, J. A. M. A. **151:** 785, 1953.
16. Vaughan, W. T., and Black, J. H.: Practice of Allergy, ed. 3, St. Louis, 1954, The C. V. Mosby Company, pp. 153, 836-840.
17. Smith, H. L.: Buckwheat-poisoning With a Report of a Case in Man, Arch. Int. Med. **3:** 350, 1909.
18. Gelfand, H. H.: Arrested Asthma and Hay Fever: Report of a Case With Constitutional Reaction From an Intradermal Test With Mustard, Laryngoscope **44:** 817, 1934.
19. Swineford, A.: Hypersensitiveness to "Asthma Powders," J. ALLERGY **8:** 607, 1937.

20. Spies, J. R., Bernton, H. S., and Stevens, H.: The Chemistry of Allergens. I. Isolation of an Active Fraction From Cottonseed, J. ALLERGY **10**: 113, 1939.
21. Cunz, H.: Anaphylaktischer Schock nach Lappchenprobe. Heilung durch Antistin, Schweiz. med. Wchnschr. **78**: 159, 1948.
22. Sidi, E., and Longueville, R.: Choc. et Urticaire apres Intradermo a la Streptomycine, Semaine hôp. Paris **30**: 1579, 1954.
23. Baagøe, K. H.: Case of Allergic Death Due to Intracutaneous Injection of 0.1 c.c. of White of Hen's Egg, Ugesk. f. laeger **90**: 550, 1928.
24. Lamson, R. W.: So-called Fatal Anaphylaxis in Man, J. A. M. A. **93**: 1775, 1929.
25. Freedman, H. J.: Acute Anaphylactic Shock Following Intracutaneous Test for Sensitivity to Horse Serum, New England J. Med. **212**: 10, 1935.
26. Hunt, E. L.: Death From Allergic Shock, New England J. Med. **228**: 502, 1943.
27. Ouer, R. A.: Allergy to Castor Bean Meal, Ann. Allergy **14**: 367, 1956.
28. Swineford, O., Jr.: Anaphylactic Shock From Skin Testing, J. ALLERGY **17**: 24, 1946.

ALLERGY (ASTHMA) TO MONKEY PROTEIN IN POLIOMYELITIS VACCINE

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ALLERGIC reactions to heterologous animal proteins commonly occur when these proteins are injected into human beings. In many instances the antigen is the major or active agent, as when antitoxins are used in passive immunity. Less frequently, the animal protein is a contaminating element in a vaccine and has nothing to do with the major function of the vaccine. Thus, in the case of vaccines made from egg-grown viruses, serious allergic reaction may occur as a result of minor quantities of residual egg albumin.[1]

Presently the Salk poliomyelitis vaccine is being used on a very large scale. This vaccine is prepared by growing the virus in monkey kidney cells grown in an artificial nutrient substrate. Virus removed from cultures carries with it small amounts of monkey tissue protein, together with whatever protein was used as part of the nutrient substrate.[2] In the brief span of the clinical use of this vaccine, no reaction to this monkey protein factor has been reported; therefore, this rather unusual instance is described.

CASE REPORT

C. D., a 9-year-old boy whose parents were Rh positive and whose father had asthma, was receiving care in the allergy clinic for an inhalant form of allergic rhinitis and asthma. His allergy background dated to the age of 4, when his parents noted that severe conjunctivitis, rhinitis, and asthma occurred in various animal houses at the zoo. Especially marked was the reaction noted in the monkey house. Because of this reaction, zoo contacts were limited. Pollen asthma subsequently developed, and for this he was brought to the clinic in October, 1954. In November, 1955, he was given 1 c.c. of poliomyelitis vaccine by his family physician. That night asthma of moderate severity developed, along with a stuffy nose. The arm was not swollen. The asthma lasted one day and subsided. One month later a second dose of 1 c.c. of vaccine was administered. Asthma and rhinitis developed in a few hours, and this time they lasted for three days. Again local reaction was minimal. On several occasions before and since these reactions to Salk vaccine oral penicillin, 1,000,000 units per day for periods as long as seven days, was given with no untoward response.

The boy was scratch tested with a 1:10 dilution of poliomyelitis vaccine and gave an immediate wheal and flare reaction. A control test on a normal subject was negative. An intradermal test with *Macaca rhesus* serum diluted 1:5,000 gave a marked immediate reaction, whereas similar testing with horse serum 1:100 produced but a slight reaction.

DISCUSSION

Allergic reactions to antigenic materials which are not pharmacologically essential constituents of a drug have been reported previously.[1] Essentially,

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such items are present in only small amounts; however, in highly allergic persons, even then serious reactions can occur. Requirements for commercial vaccine are such that antigenic sera added to a virus-monkey cell mixture as part of the nutrient substrate shall not exceed 1:1,000,000 in the final product.[3] This material (not necessarily monkey serum), plus monkey cell material carried over into the vaccine, constitutes the antigen presumably involved in the present report.

Our review would suggest that here we had an allergic child who developed a high degree of allergy to the monkey, presumably as a result of zoo exposures. Five years later the injection of poliomyelitis vaccine containing small amounts of monkey protein caused an allergic response.

SUMMARY

A rather unusual case of allergy (asthma), presumably caused by minute amounts of monkey protein contained in Salk vaccine, is presented. This situation will probably be seen only rarely, but the possibility of similar reactions, particularly with repeated injections of the vaccine, must be borne in mind.

REFERENCES

1. Plotz, H., Coulson, E. J., Stevens, H., and Stull, A.: J. Immunol. 53: 315, 1946.
2. Public Health Service Technical Report on Salk Poliomyelitis Vaccine, U. S. Department of Health, Education and Welfare, June, 1955, p. 30.
3. Ibid.: Appendix E.

STUDIES IN PENICILLIN HYPERSENSITIVITY

IV. Antigenic Properties of Altered Procaine Penicillin

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REAGINS to penicillin have been found in the sera of many patients following allergic shock reactions produced by this antibiotic. We[1] found reagins in eleven successive cases of severe immediate allergic shock reactions resulting from penicillin administration. There are, however, occasional reports of failure to find antibodies to penicillin in similar cases. It is the purpose of this article to present experimental findings which may account for such negative reports.

This study began with the investigation of Patient M. F., a 60-year-old housewife, who on April 18, 1954, experienced an almost fatal allergic shock reaction following an intramuscular injection of 300,000 units of procaine penicillin G (Pfizer), which had been given for a sore throat. Within a few minutes, the patient developed severe generalized pruritus, dyspnea, asthmatic breathing, and loss of consciousness. Epinephrine was administered and the patient was hospitalized. After several hours, she began to improve. She eventually recovered with no untoward sequelae.

This patient, who gave a negative family history for atopic illnesses, had developed bronchial asthma at the age of 45. The attacks were severe, requiring injections of epinephrine for relief of symptoms. Skin tests revealed only a positive reaction to wool. The asthma improved when the patient stayed in a warm climate. At the age of 50, she underwent a tonsillectomy and adenoidectomy. Subsequently, the asthmatic attacks gradually subsided and eventually disappeared.

Since 1947, M. F. had received many injections of penicillin without untoward reactions. The last of these had been given approximately four months before the allergic shock reaction.

Direct intracutaneous tests with penicillin were not performed on this patient. It has been our[1, 2, 3] experience and that of others that patients who have recently had anaphylactic reactions to penicillin are prone to develop similar reactions following skin tests with this antibiotic. We have consistently demonstrated circulating reagins to penicillin in these cases, although at times the reagins have been found to be transient, disappearing from the circulation within several months after the last clinical reaction.[1] In such highly sensitive patients, with reaginic sensitivity to penicillin, a safe dilution for direct skin testing has not been established. Moreover, the presence of a positive skin reaction, when elicited by direct test, does not in itself establish the presence or absence of circulating reagins. Thus, it has

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been our practice to employ the passive transfer technique, not only because it is a nonhazardous procedure, but also because it permits the detection of reagins (circulating antibodies) to penicillin in these patients.

In preliminary passive transfer studies made with the patient's serum, tests with crystalline penicillin G in concentrations up to 50,000 units per milliliter yielded negative results. This was surprising in view of the fact that in all the sera of our previous cases, positive passive transfer reactions were demonstrated with this form of penicillin, often in higher dilutions than 50,000 units per milliliter. In the light of this apparent discrepancy, further studies seemed indicated in the search for the agent which was responsible for the shock reaction in this patient.

MATERIALS AND METHODS

Sera for passive transfer studies were obtained from bleedings taken six days and one month after the allergic shock reaction. According to the technique of Walzer[4] and Bowman, sites on the arms of normal recipients were prepared by the intracutaneous injection of 0.05 ml. of serum. After a four-day interval, the sensitized sites and adjacent untreated cutaneous areas were tested with approximately 0.01 ml. of various types of penicillin solutions or other materials. After fifteen minutes, the reactions on the test and control sites were read and compared. They were graded from negative to four plus, according to the size of the wheal. The two samples of serum showed similar responses to the test materials and all results were therefore combined.

The thick consistency and irritating properties of procaine penicillin suspension rendered it unsuitable for intracutaneous testing. This was also true of the undiluted supernate obtained by centrifuging the procaine penicillin suspension. A more satisfactory preparation for skin testing was obtained by diluting the supernate with nine parts of physiologic saline. Although the irritative properties of the test allergen were considerably reduced by this dilution, nonspecific plus-minus to one-plus reactions were still occasionally obtained on the control sites with the test material.

I. PRELIMINARY STUDIES WITH VARIOUS PENICILLIN PREPARATIONS

The initial passive transfer studies with serum M. F. in which sites were tested with aqueous potassium penicillin G, in concentrations up to 50,000 units per milliliter, produced consistently negative results.

Passive transfer sites were prepared with serum M. F. on four normal subjects and were tested with the following substances: (1) potassium penicillin G; (2) various brands and lots of procaine penicillin G suspension (Pfizer, Lilly, Sharpe & Dohme, and Commercial Solvents Corporation (C.S.C.); (3) the diluent of procaine penicillin G (Pfizer); and (4) a 1 per cent procaine hydrochloride solution.

The results of the tests with the procaine penicillin G suspensions varied (Table I). Four of these suspensions gave positive passive transfer reactions

(Pfizer #JKL 538098 and Sharpe & Dohme #L 7972, #L 7920, and #1123 M). Three gave negative reactions (Pfizer #42083, Lilly, and C.S.C. #BAE 113). Potassium penicillin G, the diluent of procaine penicillin G, and the 1 per cent procaine hydrochloride consistently gave negative reactions (Table I).

TABLE I. PASSIVE TRANSFER REACTIONS ON SERUM M. F. SITES TO VARIOUS PENICILLIN PREPARATIONS

SUBSTANCE TESTED	SUBJECT A		SUBJECT B		SUBJECT C		SUBJECT D	
	TEST	CONTROL	TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
Potassium penicillin G solution—Squibb (50,000 units/ml.)	0	0	0	0	±	±	0	0
Procaine penicillin G suspension—Pfizer #42083	0	0	0	0				
Procaine penicillin G suspension—Sharpe & Dohme #L 7972	++	+	++	0	+±	0	+	0
#L 7920					+++	±		
#1123 M					+	0		
Procaine penicillin G suspension—Pfizer #JKL 538098	++	+	++	0	+++	±	++++	0
Procaine penicillin G suspension—Lilly Duracillin	0	0						
Procaine penicillin G suspension—Commercial Solvents Corporation #BAE 113	±	+						
1% Procaine hydrochloride			0	0	0	0		
Diluent of procaine penicillin G—Pfizer	0	0	0	0	0	0	±	±

Testing preparations of procaine penicillin suspensions were made by centrifugation of the suspension and dilution of the supernate with nine parts of isotonic saline.

II. EFFECTS OF AGING AND HEAT ON ANTIGENICITY OF PROCAINE PENICILLIN G

In the search for an explanation for the inconsistent results obtained with the various procaine penicillin preparations, the effects of aging under various conditions were investigated. A suspension of procaine penicillin G (Pfizer #43870) and a supernate obtained from that specimen were incubated for ten days at 56° C. A portion of another lot of procaine penicillin G (Pfizer #42083) was treated in a similar manner and another portion was placed in a physician's bag which was carried on daily calls and rounds for one month. A solution of 1 per cent procaine hydrochloride solution and another of potassium penicillin G containing 300,000 units per milliliter were also incubated with the above specimens. Passive transfer tests were then made with the original preparations and with the treated samples of these same preparations.

The two untreated procaine penicillin suspensions produced negative reactions. These same preparations, after incubation at 56° C. for ten days, elicited three-plus (+++) to four-plus (++++) positive passive transfer reactions (Table II).

As a result of incubation, the suspensions changed in color from white to amber, with a reduction in the amount of suspended material. The incubated solutions became more irritating to the skin. Control tests on normal

skin with these solutions originally produced negative reactions; after incubation, these solutions gave plus-minus (±) or one-plus (+) positive reactions.

The incubated supernate of suspension Pfizer #43870 produced a one-plus (+) passive transfer reaction and a plus-minus (±) reaction at the control site. This weak reaction with incubated supernate suggested that the change in the antigenicity of procaine penicillin suspension was not due primarily to an alteration in the small amount of dissolved procaine penicillin in the supernate. It seemed likely that the altered antigen found in solution in the supernate following incubation of the whole suspension was derived largely from the procaine penicillin crystals.

TABLE II. PASSIVE TRANSFER REACTIONS ON SERUM M. F. SITES TO PENICILLIN ALTERED BY AGING AND HEAT

SUBSTANCE TESTED	FRESH		ALTERED BY			
			INCUBATION AT 56° C. FOR 10 DAYS		STORAGE IN DOCTOR'S BAG FOR ONE MONTH	
	TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
Procaine penicillin G suspension—Pfizer #42083	0	0	+++	+	++	+
Procaine penicillin G suspension—Pfizer #43870	0	0	++++	+		
Procaine penicillin G supernate—Pfizer* #43870	0	0	+	±		
1% Procaine hydrochloride	0	0	0	0		
Potassium penicillin G (300,000 units/ml.)	0	0	0	0		

Testing preparations of procaine penicillin suspensions were made by centrifugation of the suspension and dilution of the supernate with nine parts of isotonic saline.

Each suspension of procaine pencillin contained 300,000 units per milliliter.

*The supernate of procaine penicillin suspension (Pfizer) is said to contain approximately 1.5 mg., or approximately 2500 units per milliliter of procaine penicillin dissolved in the diluent.

The suspension of procaine penicillin G (Pfizer #42083) which was carried in a physician's bag for one month and which originally had given negative transfer reactions produced a positive transfer reaction at the end of this period. It gave a two-plus (++) reaction on a sensitized site and a one-plus (+) reaction at the control site.

The incubated solutions of 1 per cent procaine hydrochloride and of the potassium penicillin G showed no changes in antigenic properties and continued to give negative passive transfer reactions.

III. FURTHER STUDIES ON THE EFFECTS OF AGE AND TEMPERATURE

The effects of temperature on the antigenicity of penicillin were studied in greater detail. Samples of the most recently prepared stocks of penicillin were obtained directly from pharmaceutical companies. Included among these were procaine penicillin G suspension (Pfizer #40190 and Lederle #3865-R570), procaine penicillin G crystals (Pfizer #40748), potassium penicillin G crystals (Squibb #3M70102), potassium penicillin O (Cer-O-Cillin, Upjohn #CF585), and benzathine penicillin G suspension (Bicillin, Wyeth

TABLE III. PASSIVE TRANSFER REACTIONS ON SERUM M. F. SITES TO PENICILLIN PREPARATIONS KEPT AT VARIOUS TEMPERATURES FROM ONE TO FOUR WEEKS

SUBSTANCE TESTED	FRESH		ALTERED BY													
			REFRIGERATION, 6° C.		ROOM TEMPERATURE, 20° C.				INCUBATION AT 37° C.				INCUBATION AT 56° C.			
			4 WEEKS		2 WEEKS		4 WEEKS		2 WEEKS		4 WEEKS		1 WEEK		3 WEEKS	
	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
Procaine penicillin G suspension—Pfizer #40190	±	±	±	0	0	0	+	0	+	±	++	±	++++	+	++++*	+
Procaine penicillin G suspension—Lederle #3865-R570	±	0	+	0	0	0	+±	0	±	0	+++	0	++++	+		
Procaine penicillin G crystals—Pfizer #40748 (suspended in saline)	0	0	0	0	0	0	+	0	+	±	+++±	0	++	0	++++	±
Potassium penicillin G—Squibb #3M70102 (dissolved in saline)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potassium penicillin O (Cer-O-Cillin)—Upjohn #CF 585 (dissolved in saline)	0	0											0	0		
Benzathine penicillin G (Bicillin suspension)—Wyeth #2540838	0	0											+	0		

Testing preparations of procaine pencillin suspensions were made by centrifugation of the suspension and dilution of the supernate with nine parts of isotonic saline.

All suspensions and solutions of penicillin contained 300,000 units per milliliter, except potassium penicillin 0 which contained 200,000 units per milliliter.

T = Test site.

C = Control site.

*Positive passive transfer reaction obtained with 1:1,000,000 dilution.

#2540838). Sufficient physiologic saline was added to the crystalline preparations to make their concentrations comparable to those of the suspensions, that is, 300,000 units per milliliter. The penicillin preparations were tested on passively sensitized sites and then divided into four parts which were stored at 6°, 20°, 37°, and 56° C. Samples of each specimen were tested at various intervals during the processing period. Sterility tests were performed on all specimens to rule out bacterial or fungal growths as a factor in the experiments.

It was found that procaine penicillin G crystals (Pfizer #40748), freshly suspended in saline, gave no positive passive transfer reactions when refrigerated for one month or when kept at room temperature for two weeks (Table III). When samples of this suspension were kept at room temperature for four weeks, a one-plus (+) transfer reaction was obtained. After incubation at 37° C., a one-plus reaction (+) was obtained at two weeks and a much stronger one at four weeks (+++±). After incubation at 56° C., a two-plus (++) reaction was obtained at one week and a four-plus (++++) reaction at three weeks. Similar results were obtained under the same experimental conditions with two suspensions of procaine penicillin G (Lederle #3865-R570 and Pfizer #40190) (Table III). After incubation at 56° C. for three weeks, procaine penicillin G (Pfizer #40190) produced a positive passive transfer reaction in saline dilutions as high as 1:1,000,000.

Potassium penicillin G and potassium penicillin O showed no changes in their antigenic properties following incubation. Benzathine penicillin G gave a one-plus (+) reaction after incubation for one week at 56° C. In its original state it had produced a negative reaction.

DISCUSSION

Considerable evidence has now been accumulated to indicate that the immediate allergic shock reaction to penicillin is mediated by a reaginic mechanism. We have found reagins to penicillin or its derivatives in every one of eleven cases which we have had the opportunity to study thoroughly. Some of the reported failures to find reagins in similar cases may now be explained. One possibility previously demonstrated by us[1] is that reagins can disappear so rapidly after the occurrence of a shock reaction that passive transfer tests performed after an interval of several weeks give negative reactions.

Another possible explanation is presented in this study. A nonallergenic suspension of fresh procaine penicillin G can become an extremely potent antigen when aged or incubated for several weeks. In tests with serum M. F., a 1:1,000,000 dilution of incubated procaine penicillin G gave positive passive transfer reactions while the concentrated fresh penicillin was inactive. There is little doubt that the allergic shock reaction experienced by Patient M. F. was caused by such an altered procaine penicillin.

The nature of the change which occurs in the procaine penicillin molecule as a result of aging is not known at this time. It is of interest that the separate components of this compound—namely, procaine and potassium penicillin G—failed to develop antigenic activity with aging. Incubated benzathine penicillin G cross-reacted slightly with altered procaine penicillin G, while potassium penicillin O did not. It may be significant that both benzathine and procaine penicillin contain two amine radicals, while the other types of penicillin tested contain only one such radical.

In view of these experiments, there is reason to question the advisability of storing procaine penicillin suspension at room temperature, as is now recommended by the manufacturers, or of keeping it in the physician's bag for prolonged periods of time. During the summer months, the rear compartment of an automobile, where the bag is usually kept, may act as an incubator. Since the experimental evidence suggests that refrigeration of procaine penicillin does not promote alteration of its allergenic properties, this type of storage would appear to be preferable.

The above experiments also cast considerable light on the limitations of the present skin-testing techniques for determining the presence of an allergy to penicillin. A negative reaction to potassium penicillin G or to fresh procaine penicillin G does not rule out the possibility of a sensitivity to altered procaine penicillin G. Under these circumstances, if tests for penicillin sensitivity are performed, the material to be injected is the test substance that should be employed. This subject will be discussed at greater length in a subsequent communication.

SUMMARY

1. The serum of a patient who developed an immediate allergic shock reaction following an injection of procaine penicillin G contained no reagins for potassium penicillin G or fresh procaine penicillin G. In this serum, reagins for procaine penicillin G which had been altered by aging and heating were demonstrated.

2. Fresh nonallergenic suspensions of procaine penicillin G became actively allergenic after standing for four weeks at room temperature, for two weeks at 37° C., and for one week at 56° C.

3. Refrigeration retarded the alteration of procaine penicillin. This method of storage is to be preferred to storage at room temperature or in the physician's bag, as is a common practice.

4. All allergic shock reactions to penicillin, in our experience, have been based upon a reaginic mechanism.

5. There are two possible explanations for failure to demonstrate reagins to penicillin in such cases. Since reagins to penicillin may disappear from the circulation within a few weeks after a shock reaction, improper timing of the test for reagins may explain some of the failures. Furthermore, reagins may be present to *altered* procaine penicillin G and not to crystalline penicillin G, as was the case in the studies herein presented.

We wish to express our appreciation to Chas. Pfizer & Co. and to Sharpe & Dohme, Inc., for providing the necessary materials.

REFERENCES

1. Siegel, B. B., Coleman, M., Weissman, L., and Scherr, M. S.: Studies in Penicillin Hypersensitivity. I. Immunologic Findings in Two Penicillin-Sensitive Patients. (To be published.)
2. Coleman, M., and Siegel, B. B.: Studies in Penicillin Hypersensitivity. II. The Significance of Penicillin as a Contaminant, J. ALLERGY **26**: 253, 1955.
3. Coleman, M., and Siegel, B. B.: Studies in Penicillin Hypersensitivity. III. The Influence of Parenterally Administered Antihistamines on Contralateral Passive Transfer Reactions to Penicillin, J. ALLERGY **27**: 27, 1956.
4. Walzer, M., in Cooke, R. A.: Allergy in Theory and Practice, Philadelphia, 1947, W. B. Saunders Company, p. 508.

American Academy of Allergy

PRESIDENT'S ADDRESS

CARL E. ARBESMAN, M.D., BUFFALO, N. Y.

FOR the past few years it has been the custom for your president to address the Academy at this luncheon, and to report on the activities of our organization during the past year. Serving as president of the Academy has been a great privilege and the highest honor that could be bestowed upon me. Hence, it is with extreme appreciation and humility that I speak with you on this occasion.

Although the presidency of the Academy demands much of one's time and presents problems, these difficulties are more than compensated for by the help of so many eminent and memorable people. I have certainly enjoyed this past year, and I have learned a great deal about being an executive of such a large and outstanding organization. As you know, as president, I was privileged to give many scientific talks to various organizations throughout the country. This requires considerable preparation, and in the course of this preparation I have increased considerably my knowledge of the various subjects upon which I spoke. It is surprising how much we take for granted. This year also leaves me greatly enriched in knowledge and friendships as a result of meeting so many of you nice people throughout the country and exchanging ideas—knowledge and friendships which I would otherwise never have obtained.

This has been a most productive and exciting year for allergy and for the Academy, but there was also one sad note. This was the great loss which the Academy suffered in the death of Dr. W. C. Spain, a secretary and former president of the Academy.

I wish, at this time, to thank the members of the Executive Committee and all other committees for their hard work and wonderful cooperation. Without the efforts of all the members, and especially those of the Executive Office, the success of this meeting would not have been possible. Particular recognition

Presented at the thirteenth annual meeting of the American Academy of Allergy, Los Angeles, California, Feb. 4-6, 1957.

should be given to Dr. Criep and his Postgraduate Committee, the Program Committee, and all the participants of the Postgraduate and Scientific Session for the fine job that has been done and is being continued through tomorrow.

Special commendations should be given for the excellent work which your next president, Dr. William B. Sherman, has done as editor of the JOURNAL OF ALLERGY for the past six years, and to Dr. Max Samter, your president-elect, for his devotion and hard work as secretary of the Research Council. Both of these fellows are leaving their posts for other duties in the Academy. Their very able successors are Drs. Lowell and Matthews, respectively.

My thanks also go to Dr. Targow, Chairman of the Host Committee, and Mrs. Targow, who had charge of the women's activities, for their fine work in planning the interesting and enjoyable entertainment. In addition, I want to thank the Schering Corporation for the very nice cocktail party given last night.

The presence of the large number of eminent guest speakers for both these sessions was made possible through a very generous grant from the Ciba Company.

Now, what has been so unusual in allergy this past year?

First, it was the fiftieth anniversary of allergy. A testimonial dinner honoring Dr. Bela Schick took place in New York City in May, 1956. The Academy's official representatives were Drs. Sherman, Chobot, and Harkavy.

Second, it was also the fiftieth anniversary of the Food and Drug Administration, and a celebration of this function was held in Washington, D. C., in June, 1956. The Academy was one of the very few medical groups that had representatives there. Drs. Grafton T. Brown, Eloise Kailin, Harry S. Bernton, Henry Stevens, and I attended this affair. A souvenir booklet with the Academy's name and its representatives is in the Executive Office. All of you have probably seen the scientific exhibit here on the Food and Drug Administration.

Third, this was the first year in which the National Institute of Allergy and Infectious Diseases, as such, was activated. After considerable "politicking and lobbying" by many interested persons, including your president and several members of the Academy, the extramural budget of this Institute was increased from approximately $2,250,000.00 to more than $8,000,000.00. (This was very exciting business.) What has happened to this money? In a report received from Dr. Karel, chief of Extramural Programs, as of Dec. 11, 1956, $8,762,298.00 for 761 grants had been allocated by the Institute for research in universities, medical schools, schools of public health, hospitals, and other institutes. Of this amount, $1,131,142.00 for seventy-three grants was given for research work in allergy. This was 9.6 per cent of the total number of grants, but 13 per cent of the total amount of money. This is considerably more money than had ever been spent previously for research in allergy. As you know, this same Institute deals with many other specialties, including infectious diseases, parasitology,

serology, tropical medicine, virology, and immunology. Hence, allergy is certainly getting its fair share from the Institute. If we were to add the additional $3,000,000.00 approved for grants in immunology and subjects related to allergy (in all forms), we would have over $4,500,000.00 for research in allergy and immunology.

The individual grants in allergy, which varied from $1,150.00 to $64,000.00, covered subjects of all types, from enzyme chemistry to air-borne allergens. Certainly, research in allergy has made a step forward, and much should come of all this.

In addition to the extramural grants of the National Institute of Allergy and Infections Diseases, an intramural program was started on Jan. 1, 1957. Dr. Jules Freund is chief of the Laboratory of Immunology. At present there are twelve staff members, but a sizable increase will be made next year. The present studies are with fundamental problems related to hypersensitivity. As the program develops, studies will be made in clinical allergy and other areas, such as immunochemistry and nonspecific mechanisms.

Although this is all very wonderful news, I feel that medicine, our specialty included, should not be completely subsidized by and dependent on the National Government. Private sources of funds for research and investigation should still be sought through various industries and foundations. We all have a stake in this. There should be a close liaison between the public funds and private organizations. We are fortunate, indeed, that we have such a liaison unit in the American Foundation for Allergic Diseases.

Fourth, during the past year the Academy gave another $2,500.00 in support of the American Foundation for Allergic Diseases. Besides engaging in public education by the publishing and distribution of the excellent booklets on hay fever and asthma and other lay literature, the Foundation this year has (1) made a survey of undergraduate and graduate training in allergy in our medical schools; (2) granted ten $500.00 stipends to medical students during the past summer for work in clinical allergy and in the basic sciences related to allergy; and (3) awarded two predoctoral fellowships of $2,500.00 and one $1,500.00 part-time fellowship.

The Foundation has many excellent plans for the future with regard to public education, training of medical students and graduates in allergy, fellowship training in allergy, patient care, and research.

Fifth, the Joint Committee on Certification, which has been under the very stimulating leadership of Dr. George Piness for so many years, deserves the utmost gratitude from the entire membership. They appeared before the Advisory Board of Medical Specialties in February, 1956. This Board recommended the formation of an Advisory Council in Allergy, made up of three representatives from the Sub-Board of Allergy in Internal Medicine, three from the Sub-Board of Allergy in Pediatrics, a representative of the Academy, a representative of the College, and one from the Section of Allergy of the A.M.A., if and when established. (I might add that there were over twenty good abstracts presented for consideration for the Session on Allergy for the A.M.A.

this year. Certainly, we would have ample material for a section rather than a session.) The function of this Advisory Council in Allergy is to study the advisability of an independent Board of Allergy within the framework of the Board of Medical Specialties. This, in our opinion, is a very encouraging step toward our goal.

Sixth, the problem of amalgamation is undergoing very careful studies by a joint committee of the Academy and the College. There are many problems, and consequently progress is very slow. As many of you remember, it took many years for our two parent societies to form our present Academy.

Another item of interest in the activities of the Academy was the Third European Congress of Allergology and the formation of the European Academy of Allergy in Florence, Italy, in September, 1956. This Congress was attended by Drs. Bram Rose and Leslie Gay, as representatives of the American Academy of Allergy.

We are very fortunate in having with us Dr. Halpern, of Paris, France, and Dr. Samuel Feinberg, president-elect and president, respectively, of the International Association of Allergology. As you know, the next meeting of this organization will be held in Paris, France, in October, 1958. Make your plans now to attend, so that the Academy will have a good representation there.

Perhaps we have been spending too much time on the political and organizational phases of allergy, and not enough time on some very practical and fundamental scientific problems that have been left unanswered for many years. Should we not re-evaluate the specialty of allergy?

You all heard Dr. Brown's excellent review of the history of Allergy this morning. What have we, as allergists, contributed in the last few years toward a better understanding of many allergic problems? True, the advent of the antihistaminics and then of the corticosteroids has helped to relieve more patients, but as yet these substances have not helped us with the basic problems. (We do not even know how the steroids and antihistaminics work!)

A few months ago I was the guest speaker at the twentieth anniversary observance of the Michigan Allergy Society. At this dinner the secretary reviewed the topics that were discussed over this twenty-year period: (1) allergy and infection—the problem of bacterial allergy; (2) the role of psychiatry and allergy; (3) atopic eczema; (4) the postragweed season syndrome; (5) drug allergy; (6) food allergy—value of skin tests versus elimination diets.

We are still discussing these same subjects without much more knowledge today.

Extravagant claims and unusual procedures have often brought justifiable criticism of our specialty. What are some of our common and practical problems that make the field of allergy so bewildering and, to the outsider, so mystifying? I shall try to mention what I feel should be strived for in order to "clean up our own house."

1. *Standardization of Nomenclature.*—This is one of the most important basic problems in allergy. In the field of allergy, there has been a great deal

of difficulty in getting qualified experts to agree on this and other related subjects. During the past year, a committee headed by Dr. Lowell was appointed to study a method of nomenclature for only one condition, namely, bronchial asthma. So far, there have been as many divergent opinions as there have been members of this committee. Multiply this by the number of persons interested in allergic conditions and by the various allergic conditions which they treat, and you see that we really have a "Tower of Babel." We do not have a common language in our specialty.

2. *Standardization of Extracts.*—As you all know, we have no accepted standard for potency of extracts. True, several years ago the Academy recommended that the phosphotungstic acid nitrogen unit be used as a temporary standard, but it has not been universally used or accepted. Many still use the weight-to-volume standard; others use the total nitrogen; and still others employ the so-called Noon unit. The various commercial houses also use varied methods of standardization for potency. Of course, we all know that an absolute standardization cannot be made until we have determined the active principle, and a chemical test is probably not the answer to this problem. Some biologic assay will undoubtedly be the best means of approaching this problem. In 1939, in an article published in the JOURNAL OF ALLERGY on assay of pollen extracts, it was recommended that the in vitro neutralization technique of reagin be used as a possible means of standardization. This, of course, would require a standard pooled, lyophilized reagin for each of the many allergens used. This makes it impractical. The natural question to ask regarding this method is: "Is the neutralization property of the extract a measure of its allergic potency?"

Other biologic assays have potential possibilities. Could the inhibition of precipitin tests with an antiragweed rabbit seurm used as an indicator or the inhibition of complement fixation be utilizable for this purpose? Even more recently, hemagglutination studies of human sera by direct means and inhibition studies of antiragweed rabbit sera with human atopic sera have been purposed as possible means of standardization by Coombs and his co-workers in England. We, in Buffalo, have also been working on this problem of hemagglutination and hope to contribute some data in the future. Again, however, is the hemagglutinating property of the antigen the same as its clinical potency? You will hear a report on hemagglutination from Dr. Rose and his group tomorrow morning.

Very recently, Dr. Rose Augustin, of the Wright-Fleming Institute in London, has reported on the use of diffusion gel technique for standardization of pollen extracts.

3. *Standardization of Skin Tests.*—To add further to our present state of confusion, we do not even agree on methods of skin testing. The clinics and clinicians in the Eastern part of the United States, for the most part, use intradermal tests almost exclusively. However, others in the Midwest, South, and Far West always screen first with scratch tests. These latter proponents feel that the intradermal tests are not safe and give many false-positive reactions.

You will hear Dr. Sherman's report in answer to this tomorrow. The intradermal advocates claim that with scratch or prick tests alone one might miss some very important diagnostic points.

4. *Standards for Preparation of Allergens.*—Our methods of preparation of allergens are indeed variable. Among the various extracting media, buffered saline solutions, glycerinated extracts, and dextrose extracts have many advocates. In a recent survey by the Food Committee of the Research Council of the Academy, it was found that the preparation of food extracts varies considerably from clinic to clinic. With all of these disputable points, it is very difficult to set up standards for strength of materials for skin testing and for schedules of dosage for treatments.

5. *Standardization of Treatment.*—Another variable is in the amount of treatment given. Some like small intracutaneous injections and, at the opposite extreme, others prefer massive subcutaneous dosages. What do we do when we give specific hyposensitization therapy? Are we satisfied that the production of a blocking antibody is the whole story? These problems, of course, are very practical. A patient transferring from one city to another for continual treatment, or to another physician in the same city, cannot receive uninterrupted care unless the first physician supplies the patient with the material he has been receiving. These are some of the mystifying aspects to which our nonallergy colleagues object.

I would like to see, as I am sure all of you would, a group of the above standards set up on some temporary basis, until the actual problems are solved so that we can all be speaking the same language. Standards such as those set up by the American Heart Association would be most welcome for our specialty. This could be started by our own committee of the Academy, which then could work with committees of such similarly interested groups as the College, the Foundation, and the Institute.

I have dwelt at great length on this problem because I feel that it is one of the functions of the Academy to organize our views in allergy.

There are many other clinical problems which need very urgent attention. We, as physicians, must primarily relieve pain and suffering, as well as prevent them. It should be the function of the Academy to exchange information and knowledge on all problems related to allergy.

Perhaps the reason that we are not making much progress with the problems of a clinical nature and those of standardization mentioned earlier is that we need new and different approaches to help us with our difficulties. One reason why we invite men in allied fields to talk to us is that, perhaps, we can utilize their knowledge and techniques to further advance our own specialty. We must look not only to the basic sciences, but also to the careful observations of our clinicians for solutions to these problems.

Recognition of our specialty as an entity of its own within the framework of organized medicine should be achieved within a few years. Let us deserve it! The opportunities for investigation are multiple and the exploration of these

problems has been made more attainable through help from the National Institute of Allergy and Infectious Diseases, the Foundation for Allergic Diseases, and other private funds. Let us all take advantage of these opportunities to help put allergy in its rightful place in medicine. The scope of the allergist will be broadened as we learn more about the collagen diseases, kidney disease, rheumatic fever, tissue sensitivity, etc.

I trust that this message is taken in the spirit in which it is given—not as a reprimand or criticism of our specialty, but rather as a self-analysis, as I see it, and as a challenge for all of us.

Erratum

In the March issue, in the article entitled "Sensitivity to Hair Dyes" by Reiss, Gahwyler, and Lustig, the last sentence on page 139 should read: "Of the eight persons found sensitive, four (0.39 per cent) reacted only to the black hair dye based on paraphenylenediamine, three (0.29 per cent) reacted only to the black hair dye containing paratoluylenediamine, and one (0.10 per cent) reacted to both dyes." Also, the name of the second author is Max Gahwiler instead of May Gahwyler.

HISTORIAN'S REPORT

THE thirteenth annual meeting of the American Academy of Allergy was held at the Hotel Statler, Los Angeles, California, Feb. 4, 5, and 6, 1957. This was preceded by a preconvention scientific assembly on February 2 and 3.

There were 105 members, 154 Fellows, and 183 guests in attendance. Nine came from Canada, two from Cuba, one from Finland, one from France, one from the Canal Zone, one from Hawaii, and one from Mexico. Approximately 300 ladies registered. There were 166 exhibit personnel staffing the forty-five commercial exhibits. There were eight scientific exhibits.

The scientific program was composed of thirty-one papers by Academy members and others representing original work in both investigative and clinical allergy. One of these was given by Bernard N. Halpern, an Honorary Fellow, from Paris. There were addresses by Peter H. Forsham, M.D., University of California School of Medicine ("The Dynamics of Adrenal Cortical Activity in Man and Their Relation to Hypersensitivity"); Russell Weiser, M.D., University of Washington School of Medicine ("Mechanisms of Immunologic Tissue Injury"); George W. Beadle, Ph.D., D.Sc., California Institute of Technology ("The Genetic Basis of Biological Specificity"); Dr. Ethan Allan Brown, President, American College of Allergists ("The Changing Picture of Allergy"); John R. Cann, M.D., University of Colorado Medical Center ("Biophysical Characterization of Reaginic and Blocking Sera"); and Irving Gordon, M.D., University of Southern California School of Medicine ("Identification of Antigens and Cells by Means of Antibodies"). The appearance of the guest speakers on the program was made possible by a grant from Ciba Pharmaceutical Products, Inc. The program also included the report of the Committee on Drugs of the Research Council.

At the Business Meeting forty-nine New Members and one Affiliate Fellow were elected; twenty-nine Members were advanced to Fellowship, and David W. Talmage, M.D., was elected directly to Fellowship. The following officers were elected:

William B. Sherman, M.D., *President*
Max Samter, M.D., *President-Elect*
Marion B. Sulzberger, M.D., *Vice-President*
Francis C. Lowell, M.D., *Secretary*
George I. Blumstein, M.D., *Treasurer*
Homer A. Howes, M.D., *Historian*
Edmund L. Keeney, M.D., *Member Executive Committee*

New business included modification of the Constitution to clarify the various classes of membership and action to merge the work of the Sub-Committees

on Undergraduate and Graduate Education into one Sub-Committee to provide a more effective means of encouraging teaching in allergy.

The meeting was a very pleasant one. The weather was ideal, with warm, sunny days. Great thanks are due Dr. and Mrs. A. M. Targow, who served as Chairmen of the Committee on Local Arrangements and the Hostess Committee. The Los Angeles Allergy Society gave a cocktail party and reception at the Hotel Statler on Sunday, February 3. The Schering Corporation's cocktail party was held on Monday, February 4. There was a dinner-dance on Tuesday, February 5, at Sportsmen's Lodge.

Following the Los Angeles meeting, fifty-three members went on to a special Honolulu session, held Feb. 7 to 19, 1957.

The fourteenth annual meeting of the Academy will be held at the Bellevue-Stratford Hotel, Philadelphia, Pennsylvania, Feb. 3, 4, and 5, 1958.

HOMER A. HOWES, Historian

Editorial

TWENTY-EIGHT YEARS

DR. WILLIAM B. SHERMAN resigned as editor of the JOURNAL OF ALLERGY in February, 1957, having succeeded Dr. Harry L. Alexander in 1950. The readers of the JOURNAL owe Dr. Sherman a debt of gratitude for the excellent job he has done.

As Dr. Alexander notes in an editorial entitled "Twenty Years,"* the first issue of the JOURNAL appeared in November, 1929, at the time when the bright prospects of the "twenties" were toppling. A brief announcement on page 1 set forth the *raison d'être* of the JOURNAL and then, with praiseworthy candor, grappled with the ever-present problem of what is meant by the word "allergy." In using the word, the editors recognized that "it does not possess an established meaning in scientific usage" and they would accord to it a meaning "which corresponds with current medical usage," to wit, "conditions of specific hypersensitiveness exclusive of anaphylaxis in lower animals." The announcement was then followed by articles on asthma, the incidence and distribution of pollen and an airborne insect allergen, bacterial allergy, histopathology of nasal allergy, and classification of specific hypersensitiveness. Similar titles were destined to reappear in the JOURNAL many times in the future.

The lean years of the depression which followed the appearance of the first issue were lean years for the JOURNAL as well, but it survived. Until 1950, under Dr. Alexander's able guidance, and then under that of Dr. Sherman until February of this year, the JOURNAL has thrived and should continue to thrive in the future. It is fitting at this time to acknowledge Dr. Alexander's courage and wisdom in overseeing the JOURNAL'S birth and growth and to express appreciation and thanks to Dr. Sherman for his role in expertly guiding its further development.

*Journal of Allergy **20**: 461, 1949.

Announcements

Third International Congress of Allergology
Paris, France, October 19-25, 1958

The Scientific Program will be composed of:

I. Symposia by invited international authorities on such topics as Asthma and Emphysema, Physio-Chemical Properties of Allergic Antibodies, Recent Advances in Phases of Clinical Allergy, Biochemical Aspects in Hypersensitivity, Round-Table Conference on Auto-Immune Reactions, Allergy Management and Problems in Different Parts of the World (including socio-economic aspects), and Demonstration of Special Techniques in Clinical and Experimental Allergy.

II. Short (ten-minute) papers on any phase of allergy to be presented at sectional meetings. Those in the United States and Canada who wish to present a short paper should mail title and abstract (100 to 200 words) to *Dr. S. M. Feinberg*, President, I. A. A., Northwestern University Medical School, 303 E. Chicago Ave., Chicago 11, Illinois. Those from other countries should mail abstracts to Dr. Bernard N. Halpern, 197 Boulevard St. Germain, Paris VII°, France.

A postconvention medical tour is also being planned.

The social aspects of the Congress will be attractive, including a reception and cocktail party at the Louvre Museum, an evening at the opera (ballet), conducted tours in the surrounding country on the social day, and a farewell party at the Medical School.

The meeting will be held at the new Medical School, which has ample facilities and modern acoustics; a four-language interpreter phone system will be used. Steps are under way to obtain reduced transportation fees. Travel arrangements will be announced later.

For information on local arrangements, etc., write to Dr. Bernard N. Halpern (President-Elect), 197 Boulevard St. Germain, Paris VII°, France.

For general information, write to Dr. José Quintero Fossas (Secretary-General), Paseo 313, Vedado, Havana, Cuba.

For any other additional help or information, write to Dr. Feinberg.

You are assured of an interesting scientific and social experience.

Uruguayan Society of Allergology

The following officers have been elected for 1957:

President: Prof. Dr. Benigno Varela Fuentes
Vice-President: Dr. Pablo Recarte
Secretary: Dr. Julio A. Moretti
Pro-Secretary: Dr. Jorge Vidart Megget
Treasurer: Dr. Juan C. Dighiero
Pro-Treasurer: Dr. Luis A. Martinez Bula

Obituary

RALPH M. MULLIGAN

RALPH M. MULLIGAN, Reading, Pennsylvania, was fatally injured in an automobile accident on Feb. 25, 1957.

Dr. Mulligan was Allergist and Chief of Medicine at St. Joseph's Hospital in Reading, Pennsylvania. He also served as Secretary-Treasurer of the Pennsylvania Allergy Association over a period of ten years. A jovial and fun-loving person, Dr. Mulligan was well known to practically all the members of the American Academy of Allergy.

He was born in Uniontown, Pennsylvania, in 1904. After earning a bachelor's degree at High Point College in North Carolina, he graduated from the Medical College of Richmond, Virginia, in 1934. That same year he became an intern at St. Joseph's Hospital in Reading; he also took his residency there. Four years later he entered the field of allergy while attending the University of Pennsylvania Medical School. He furthered his education in allergy by taking courses at the Roosevelt Hospital in New York City.

He was a member of the Berks County Medical Society, Pennsylvania Medical Society, the American Medical Association, the Pennsylvania Allergy Association, the Philadelphia Allergy Association, and the International Correspondence Club of Allergy. He was a Fellow of the American Academy of Allergy and a Fellow of the American College of Allergists.

Just as impressive as his medical background was his participation in sports activities, both in Berks County and on a nationwide scale. He was a well-known personality in basketball and track circles.

The officers and members of the Academy extend their sincere sympathy to Dr. Mulligan's wife, Florence; his 19-year-old daughter, Sheila; his 17-year-old son, James C.; and his 12-year-old daughter, Beth.

S. D. L.

Book Reviews

The Physician-Writer's Book. By Richard M. Hewitt, A.M., M.D. Philadelphia and London, 1956, W. B. Saunders Company. 415 pages, 37 figures. Price, $9.00.

By his Hippocratic oath, the physician is sworn to share fully his professional knowledge with his colleagues and students. He is further encouraged by the tendency of medical groups to judge his professional ability as much by his published works as by deeds performed in his practice. For these and perhaps occasionally more selfish reasons, physicians are producing an increasing volume of writing, the publication of which requires a number of medical journals now estimated to total 4,512.

The average quality of this tremendous volume of written material is better known to the editors who see it in its pristine state than to the readers who receive it after some of the worst has been eliminated and the more obvious defects of the remainder amended. While the content is more important than the form, Alan Gregg has expressed the belief that the level of medical and scientific writing constitutes the most serious internal limitation to medical education and reseach.

Dr. Hewitt, who as senior consultant in the Section on Publications of the Mayo Clinic, has given editorial aid to many authors, writes this book in the hope of improving this state of medical writing. He guides the physician-writer from the decision of whether to write at all, through the intricacies of planning, writing, and illustrating the manuscript, to the procedure for obtaining a copyright on the finished product.

Since medical writers usually gain their audiences through professional rather than literary ability, there are few who would not benefit from careful study of this book. Editors of medical journals would welcome its inclusion among the texts prescribed in medical schools.

While it contains an abundance of valuable advice and information, this book, like many other medical writings, falls somewhat short of perfection. Since it is chiefly intended for the physician who does not have access to the services available at the Mayo Clinic, a book of this size might well devote more consideration to illustrations and to statistical methods. Some readers may feel that the author's comments on brevity and unity are more valuable than his own example in following them.

Allergy and Asthma. The Relationship Between Allergy and the Autonomic Nervous System Especially the Parasympathicus. By Masuichi Takino, M.D., and Yoshitada Takino, M.D. Tokyo, 1956, Maruzen Company, Ltd.

More than forty years have passed since Eppinger and Hess* described a form of neurosis which they called "vagotonia." Their theory was that the vagus nerve, which supplies autonomic stimulation to the internal organs, and the fifth trigeminal nerve were overactive and so gave rise to one or more of a series of typical symptoms which could develop in "vagotonic" persons. The symptoms included flushing of the skin (with hot,

*_Vagotonia: A Clinical Study in Vegetative Neurology_ by Dr. Hans Eppinger and Dr. Leo Hess of Vienna. Authorized translation by Krans, W. M., and Jelliffe, S. E., New York, 1917, Nervous and Mental Disease Monographs.

red hands), sweating, stuffiness of the nose, widening of the palpebral fissure (with dilatation of the pupil), slowing of the heartbeat, spasm of the bronchial muscles, salivation, spasm of the esophagus, intestinal cramps, and a tendency to diarrhea, any or all of which could be explained by a chronic overstimulation of one organ or another.

In many patients with asthma, bronchospasm is the only evidence of "vagotonia," and it would be unreasonable to apply the theory to all cases. Furthermore, granted that an overactive vagus is important, one must still explain why this vagus is overstimulated. We can even consider that "vagotonia" is more an effect than a cause of the fundamental process.

Be this as it may, the fact remains that in this day, when approaches to the problems of allergy and asthma are so obscure, the two doctors Takino have done well to remind us that disturbances of the autonomic nervous system should be included in our thinking.

Their 222-page book deals with the theory that increased "tension" of the "parasympathicus" (by which they mean, apparently, an overactivity or hyperirritability of the greater vagus system) can explain the symptoms.

This "tension" can be general or local; it can vary from day to day, or even from hour to hour; and it can shift from one organ to another. This change is the important item. Whereas the theory cannot be proved, it is suggested by the alternation of symptoms. It is observed that allergic disease in one organ inhibits disease in another organ. For example, asthma appears when eczema subsides. The authors believe that the "tension" is all important, and their whole theme is built around that concept.

The local parasympathetic tension alternates with the general tension. The tuberculin skin test becomes weaker in the presence of severe asthma because the "skin parasympathicus" loses its tension. Food allergens which produce asthma because the lung tension is high do not produce digestive symptoms, because the gastrointestinal tension is low. Pilocarpine excites the nerve endings of the parasympathicus; it raises the tension, and it causes bronchospasm (asthma) even in normal persons.

When the atomic bomb was exploded over Hiroshima, 212,000 people were killed and 100,000 were injured. Professor Uragi found forty-five asthmatic patients who had been exposed, and when seven of these developed keloids after their burns they became free of asthma (the skin parasympathicus had been stimulated). Likewise in leprosy, the skin lesions of the trophoneurotic type have a curative effect on the asthma which may occur occasionally in the same patient.

There are three tests by which vagotonia or increased tension of the parasympathicus can be recognized: (1) Czermak's test, which consists of pressing on one or both carotid sinuses with the finger to produce slowing of the pulse, dizziness, deep breathing, and even syncope and suffocation; (2) Aschner's test, in which pressure on the eyeballs causes slowing of the pulse (in only seven out of 200 cases, however, did this test produce asthma); and (3) the response to stroking the skin where a red line, or especially a raised line, produces dermographia in patients with a "high tension of the skin parasympathicus." The authors claim that since "dermographia rubra" is strong in tuberculosis, the fact shows that the tension of the skin nerve inhibits the tension of the lung parasympathicus to make asthma rare in this disease.

Treatment of asthma depends chiefly upon raising the tension of the "skin parasympathicus," so as to inhibit the "lung parasympathicus," and for this the authors have three preparations which can be injected into the skin to set up a local skin reaction. Unfortunately, the methods of manufacture are not described, but the general principles are set down:

> *Asthremedin* consists of "denatured protein ingredients taken from the inoculated tissue with the living virus of the cowpox lymph."
>
> *Neurotropin* is a "conjugated polysaccharide taken from the inoculated tissue with living virus of cowpox lymph."
>
> *Florads* is the "effective component taken from the fluorescent microbe which lives in the sea."

Asthremedin is the most used. It is injected intradermally two or three times a week, and the treatment may be continued indefinitely, sometimes for three years. If Asthremedin in its "Number 2" dilution causes no local reaction, the Neurotropin is substituted. Florads may be added later, for this is a "preventive medicine."

Forty-five of ninety-three cases of bronchial asthma have been "completely cured," but the duration of follow-up is not stated. If these remedies fail, then, as the authors state, "ACTH or Cortisone is very useful." But just how the action of these steroid hormones fits in with the nerve tension theory is not discussed.

As a whole, the book contains too much repetition; the material could be condensed into at least one-third of the present space. At the end are three additions: The first comprises five short papers by other doctors who have used Takino's remedies with success. The second is a paper by M. Takino himself, entitled, "The Importance of the Natural Healing Process for the Cure of Asthmatic Children," which again dwells on the importance of the parasympathetic system and its "self regulation." The third addition is a résumé, four pages long, which describes the whole book in short readable style, and this résumé is repeated in French, German, and Spanish translations.

The book suggests an interesting theory about allergy and asthma. Since the whole problem is so difficult and the approach is so obscure, it is not surprising that objective support for the theory of "nerve tension" is hard to find. The reader would like more discussion of the effects of varying "tensions" on antigen-antibody reactions but this, too, will have to await further study.

Meantime, treatment with Asthremedin helps some patients, just as nonspecific therapy of other varieties helps other patients. Whether or not the theory of shifting nerve tension is the best explanation for the good effect of treatment requires comparison with such older observations as the effect of fever on immune processes, the saturation of antibodies by nonspecific means, or the so-called "anamnestic reaction." This reviewer gains the impression that the book was completed just a little too soon.

F. M. R.